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ORIGINAL ARTICLES

THE IMPORTANCE OF THE ADRENAL GLANDS IN THE ACTION OF CERTAIN ALKALOIDS*

II. STRYCHNINE ON THE BLOOD PICTURE

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IN a recent paper by one of us (Edmunds[7]), it was shown that the adrenal glands were responsible for some of the effects which are produced in the body by the administration of certain alkaloids, such as pilocarpine, physostigmine and strychnine, which stimulate these glands to increased activity. All of these alkaloids have been shown by Stewart and Rogoff[1, 2, 3] to increase the amount of epinephrine in the blood; the first to a slight degree, the other two to a much greater extent. It became therefore a matter of much interest to see whether it would be possible to demonstrate a still wider effect of the epinephrine which is secreted under their influence and which might possibly explain some of the results which follow their administration.

One of the characteristic results of the administration of small quantities of epinephrine is the change which it produces in the total number of white blood cells and in the relative proportions of the different varieties. These changes have been described by several writers and are the subject of an extended study by Edmunds and Stone.[4] Briefly stated, in dogs the effect upon the blood of small doses of epinephrine is as follows: immediately following the injection the total number of leucocytes is increased, both lympho-

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The companion paper upon the effect of physostigmine upon the blood picture will appear in a subsequent number of this Journal.

cytes and polynuclear cells being involved in the increase. After this so-called first stage, the lymphocytes decrease in number while the polynuclears continue their upward course for a variable time after which they too participate in the decline. Unessential modifications of this type of curve are common but the general characteristics described are usually present. It is true that at times from certain animals a curve differing widely from that described above is obtained, for which no adequate explanation has been offered. The effect produced in dogs by large doses of epinephrine (1 mg. per kg. body weight) is not essentially different from that of the small doses insofar as the white blood cells are concerned. However, in this case, the red cells are increased in number, this increase beginning within fifteen minutes after the alkaloid is given and lasting for about two hours. (Chart I.)

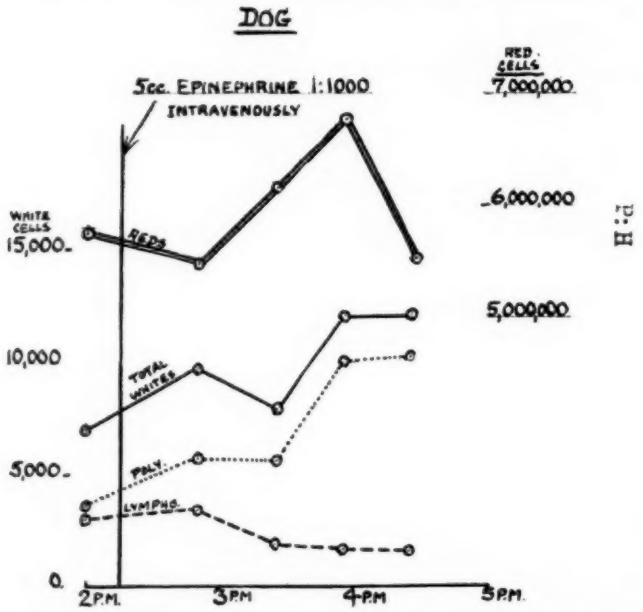

Chart 1.—Dog. Effect of administration of large dose of epinephrine.
In all charts the double line indicates the red count with the scale given at the right. Single heavy line is the total leucocyte count with scale given at the left. Dotted line is the polynuclear leucocyte count while the broken line indicates the lymphocyte count. Time is marked on the base line.

On account of the relative delicacy of the reaction of the leucocytes it was thought that it might be possible that they would react to an increase in epinephrine which would be brought about through the injection of the alkaloids named above.

The experiments were carried out essentially as in the work on epinephrine referred to, dogs being used as experimental animals and the blood obtained by a prick on the nose. Some variations in this technic will be mentioned. In the experiments where no operative procedure was necessary, a general anesthetic was not always administered but in a few animals a small injection of morphine was given to keep the animal quiet. Stewart and Rogoff[5] have shown that morphine has no effect upon the output of the

adrenal glands of dogs. In still other dogs, sodium luminal was utilized to keep the animal quiet with rather interesting results as will be shown later. The blood counts were made by the ordinary clinical methods. The spreads were stained with Wright's stain and the differential counts, as well as the counts of the red and white blood cells, were all made by one of us (Lloyd).

The action of strychnine was first studied. As is well known, Stewart and Rogoff[3] have shown that this alkaloid increases to a marked degree the amount of epinephrine secreted by the adrenal glands, even up to 10 or 15 times the normal and this even when the strychnine is given in what may be termed therapeutic doses. In our experiments, the initial dose of strychnine was 1 mg. If after a time, no increase in reflexes was found, an injection of 0.5 mg. was given, which dose was repeated if no marked symptoms

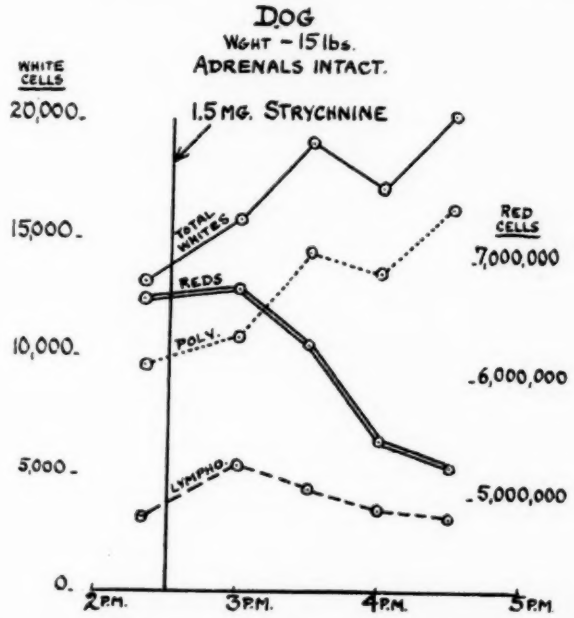

Chart 2.—Dog. Adrenals intact. Subcutaneous administration of 1.5 mg. strychnine. Compare leucocyte curves with those shown in Chart 1. Designation of curves is the same as in Chart 1.

resulted. Increased reflexes usually resulted then and in one dog a total dose of 2 mg. of strychnine was followed by a mild convulsion. The experiments were carried out upon three dogs with uniform results so that the figures and chart of only one need be given. The dog chosen for report was fully anesthetized with chloretone and paraldehyde so that it would be a fair control for the animals which on account of operative procedures had to be anesthetized similarly. The results with or without anesthesia were essentially alike.

The accompanying chart (Chart 2) shows more clearly even than the above figures the general course of a curve which in all its essential details is identical with that which would be given by a small dose of epinephrine.

It is interesting to note that on account of difficulty in getting blood from the nose in two of the counts, cuts were made through the skin over the

TABLE I

MAY 31, 1922	DOG, 15 LBS. REDS	WHITES	CHLORETONE-PARALDEHYDE POLYNUCLEAR	ARTIFICIAL RESPIRATION LYMPHOCYTES	MONONUCLEAR
2:20	6,650,000	13,160	9,700	3,200	260
2:30	1 mg. strychnine subcutaneously.				
2:40	0.5 mg. strychnine subcutaneously.				
3:00	6,790,000	15,900	10,600	5,100	200
3:30	6,290,000	19,000	14,500	4,300	200
4:00	5,480.000	17,000	13,500	3,400	100
4:30	5,240,000	20,100	16,300	3,300	500

neck and small arteries opened and the counts made from this actively running blood. This variation in technic produced no distortion of the curve

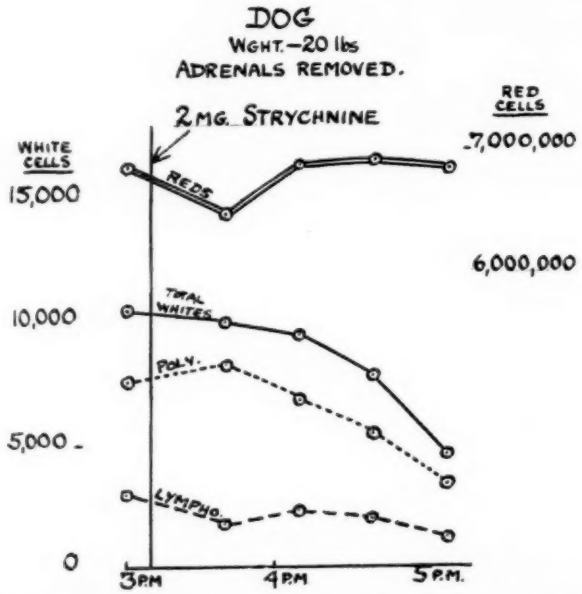

Chart 3.—Dog. Adrenal glands removed. Effect of 2 mg. strychnine. Compare leucocyte curves with those in Chart 2. Note that the red cell curve with these strychninized dogs shows no characteristic course.

which was essentially identical with that obtained from other animals where all the counts were made from capillary blood obtained from the nose. This fact, which was confirmed in other animals under similar conditions, has an important bearing upon the whole question, connected as it is with the theory that a possible unequal distribution of cells might be responsible for the changes in blood count.

In the second series of dogs, both adrenal glands were removed. The abdomen was then closed and the animal allowed to remain quiet for a considerable time in order to permit of proper readjustment of the circulation. Blood counts and spreads were then made. Strychnine was now given by subcutaneous injection as in the control animals, doses of from 1.5 to 2 mg.

being administered. Blood was taken at half hourly intervals and Chart 3 shows a curve obtained under these conditions. Other curves were similar so need not be given nor need the numerical results be cited as they are shown clearly in the chart. The course of the curve is entirely changed. Instead of an increase in leucocytes, there is a decrease. The conclusions are clear. The increase in the number of leucocytes and the change in their relative proportions which are produced in intact normal dogs by strychnine are due to the effect of the alkaloid upon the adrenal glands—in other words, they are epinephrine effects. The number of red cells, however, in these animals is not greatly altered as the amount of epinephrine produced is com-

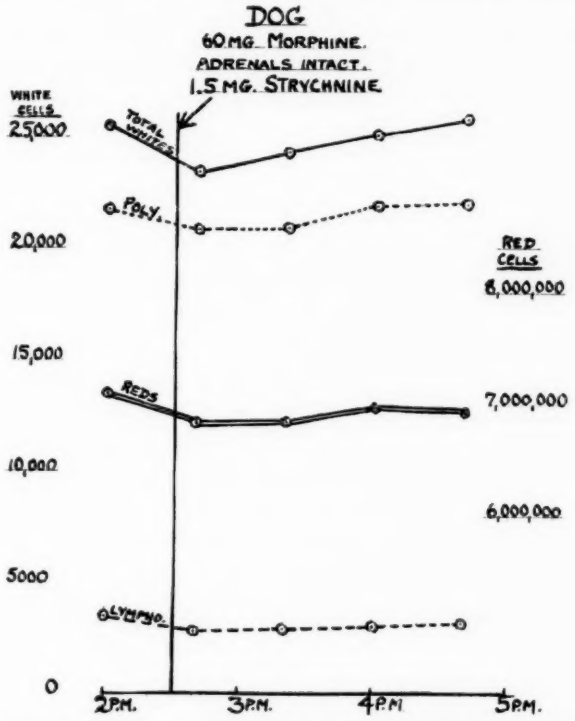

Chart 4.—Dog. Adrenals intact. Preliminary injection of morphine followed after some hours by strychnine 1.5 mg. See text for discussion.

paratively small, while to modify the number of erythrocytes a large amount of epinephrine is required as was pointed out earlier in this paper.

Two or three features encountered in these experiments deserve special mention: first, as to the effect of muscular movements which it might be thought would play a part in modifying the course of the curves. In two dogs each receiving two milligrams of strychnine, convulsions occurred. In neither case was the curve of either red cells or white cells modified. On theoretical grounds marked deviation might have been expected due to a possible squeezing of cells out of the capillaries of the muscles. This did not occur.

Two interesting variations in results were encountered due to the anes-

thetic used. In the first, morphine was employed. Now, Stewart and Rogoff[5] have shown that morphine has no effect upon the epinephrine output in dogs, but that in cats where the morphine has a stimulating effect as shown by the typical excitement, the adrenal glands are stimulated and an increase in epinephrine in the blood is demonstrable. In one of our dogs to which 60 mgs. of morphine were given, wild excitement followed, essentially the same symptoms as are seen in the cat under similar circumstances. In this animal, the reaction to the 1.5 mg. of strychnine as seen in the course of the blood cell curves was much less than in the average normal dog which is depressed by morphine; in fact, the strychnine effects may even be said to be nil as shown in the chart (Chart 4). The explanation doubtless was that this dog's adrenals were already stimulated to increased activity by the morphine and the strychnine effect was not discernible. In other words, this dog reacted to morphine after the manner of cats insofar as its central nervous system was concerned and because of that action its adrenals were stimulated to increased activity.

Another variation in curves was encountered in two dogs. Compelled by the experience just described to give up morphine as an aid in keeping our normal animals quiet, we tried sodium luminal which was recommended as an anesthetic by Drinker.[6] For our purpose, which was simply as a depressant, it was not satisfactory on account of the very severe depression which it produced. The prostration was so marked, the luminal depression of the cord so strong, that the strychnine action was greatly lessened and while epinephrine curves were obtained the changes were not so marked as in animals in which other depressant drugs were employed. In one of these dogs, the total leucocyte count rose from a normal of 14,400 to 16,300 while in the other it rose from 1ɔ,000 to 17,000 as compared with increases of 8,000 or 10,000 commonly seen in normal animals. Both morphine and luminal experiments were interesting confirmations of the localization of the strychnine effect upon the adrenals as being upon the central nervous system.[3]

CONCLUSIONS

The changes in the total number of leucocytes in the dog and in the relative proportions of the different varieties of white blood cells produced by strychnine are only indirectly due to strychnine but are directly due to the epinephrine which is secreted by the adrenal glands under the influence of strychnine. The epinephrine thus secreted is not in sufficient amount to alter the number of the red blood cells.

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HISTOLOGICAL STUDY OF MUSCLE DEGENERATION PRODUCED BY LOCAL INJECTIONS OF INSULIN*

By D. J. Bowie, B.Sc., (Med.) and W. L. Robinson, M.B.

IN the experimental and clinical use of insulin as a diabetogenic reagent we were struck with the local reactions produced in rabbits and human beings about the site of the inoculation. In the rabbit more particularly one very often found a marked local subcutaneous edema somewhat mucigenous in character and extending for a considerable distance beyond the site of the inoculation.

Histological preparations of this edematous tissue were made and stained with Mallory's eosin and methylene blue, phosphotungstic acid hematoxylin, Van Gieson and Mallory's aniline blue stains. These we studied very carefully, but were unable to find any change in the connective tissue cells nor any abnormal deposits between them of a degenerative character.

The cells were widely separated by an unstainable fluid material. Certain sections, however, which included portions of the neighboring muscles showed degenerative changes going on in some of the superficial muscle fibres. To determine the nature of this degeneration intramuscular injections of insulin were made into a series of rabbits. These were killed at various periods of time after the injection and the nature of the degeneration as well as the sequence of events studied.

Exp. 1.—Rabbit. (1-25-23A) Normal. 1 c.c. of sterile insulin injected into the left sacro-spinalis muscle after shaving and mopping the skin with alcohol. Ten minutes later the muscle tissue removed and fixed in Zenker's fluid. Study of these sections revealed no evidence of muscle degeneration. The stroma was slightly edematous, no evidence of cellular reaction.

Exp. 2.—Rabbit. (1-25-23B) Normal, as in experiment No. 1 injected with 1 c.c. of insulin into the left sacro-spinalis muscle. One half hour later muscle removed. No evidence of oedema in the gross. Sections fixed and stained as before and show some evidence of a beginning degeneration of the muscle fibres, which is characterized by an apparent autolysis of portions of the cytoplasm leaving a fine granular debris. Between these areas are irregular fragments of slightly deeper staining cytoplasm. The longitudinal and transverse striations of these latter fragments of cytoplasm are gone. There is no evidence of an inflammatory reaction. The sarcolemma is intact.

Exp. 3.—Rabbit. (1-25-23C) Normal, as in experiment No. 1 injected with 1 c.c. of insulin into the left sacro-spinalis muscle. One hour later muscle removed. Sections fixed and stained as before show fairly marked degenerative change in groups of the muscle fibres similar to that found in experiment No. 2. The sarcolemma is intact. There is no evidence of inflammatory reaction.

Exp. 4.—Rabbit. (1-4-23B) Normal, injected as in experiment No. 1 with 1 c.c. of insulin into the left sacro-spinalis muscle. Four hours later muscle removed and fixed

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in Zenker's fluid. There was a slight amount of oedema apparent in the gross. Sections stained as before show a pronounced degenerative change in some of the muscle fibres characterized by a wrinkling of the cytoplasm within the sarcolemma into coarse twisted cords and showing a loss of longitudinal and transverse striations.

EXP. 5.—Rabbit. (12-5-22C) Normal, well fed for three days previous to inoculation including glucose in its food. An injection of 1 c.c. of sterile insulin made subcutaneously into the right hind leg. Eight hours later examined. No edema but slight amount of hemorrhage. Neighboring muscle tissue fixed and stained as before show in one or two areas some of the superficial muscle fibres degenerated similarly to experiment No. 4. There is a marked infiltration of the stroma with polymorphonuclear leucocytes. Some of the muscle fibres are infiltrated with these same cells. The sarcolemma is intact.

Fig. 1.—Microscopical section of muscle near site of injection of insulin showing characteristic changes especially in Experiments 4 and 6.

EXP. 6.—Rabbit. (0-26-22) Normal, injected subcutaneously into the back with 4 c.c. of insulin. Five hours later given another injection of 4 c.c. subcutaneously into the back. Animal died twelve hours later. Marked subcutaneous edema over the back. Superficial muscles of this area fixed and stained as before show a moderate diffuse lymphocytic infiltration of the external sheath of the muscle. About one third of the muscle fibres show degeneration as described in experiment No. 4 but more extensive. Cross section of degenerated fibres show some wrinklings of cytoplasm into irregular coils with no directional tendency. The muscle fibres are affected in a very irregular manner. Sometimes two or three fibres together are degenerated, in other areas just isolated fibres show this change. Usually just a portion of any one fiber appears degenerated.

In order to determine whether this degeneration is characteristic of insulin or some toxin in its preparation a series of rabbits were injected intramuscularly with saline, glucose and adrenalin solutions as well as insulin. These were checked one against the other in the same rabbit by carefully shaving and mopping the skin with alcohol and injecting one solution into the sacrospinalis muscle and one of the other solutions in the same muscle on the opposite side. Eight hours was allowed in each case, as this gave plenty of time for the degeneration to reach its maximum. The pieces of muscle tissue removed from the site of inoculation were fixed in 10 per cent formalin, embedded in paraffin and stained with hematoxylin and eosin.

EXP. 7.—Rabbit (2-28-23A) Normal (A) 1 c.c. sterile isotonic glucose injected into left sacro-spinalis muscle. Microscopic section shows slight amount of muscle degeneration immediately about the needle puncture, probably traumatic. The muscle degeneration is apparently the same as described in experiment No. 4.

(B) One c.c. sterile hypertonic glucose injected into right sacro-spinalis muscle. Degeneration the same but much more extensive.

EXP. 8.—Rabbit. (2-28-23B) Normal. (A) One c.c. sterile hypertonic glucose solution injected into left sacro-spinalis muscle. Muscle degeneration is very extensive and marked, similar to (B) in experiment No. 7.

(B) One c.c. sterile insulin injected into right sacro-spinalis muscle. Muscle degeneration similar to, but more extensive than that of the opposite side.

EXP. 9.—Rabbit. (2-28-23C) Normal (A) One c.c. sterile insulin injected into left sacro-spinalis muscle marked and extensive muscle degeneration similar to (B) Exp. 8.

(B) One c.c. of 1-10,000 sterile adrenalin solution injected into right sacro-spinalis muscle. Very slight amount of muscle degeneration but similar to that described in experiment No. 4.

EXP. 10.—Rabbit. (2-28-23) Normal. (A) One c.c. sterile isotonic saline injected into left sacro-spinalis muscle. Small localized area of muscle degeneration about puncture wound, probably traumatic.

(B) One c.c. sterile hypertonic saline solution injected into the right sacro-spinalis muscle. Moderate amount of muscle degeneration similar to above experiments.

Exp. 11.—Rabbit. (3-7-23A) Normal. (A) One c.c. sterile 3 per cent hypertonic saline solution injected into left sacro-spinalis muscle. Slight amount of muscle degeneration confined pretty well to needle puncture wound. Partly or wholly traumatic.

(B) One c.c. of a 6 per cent hypertonic sterile saline solution injected into the right sacro-spinalis muscle. Muscle degeneration a little more extensive than that found on the opposite side.

EXP. 12—Rabbit. (3-7-23B) Normal. (A) One c.c. sterile 1-1,000 adrenalin solution injected into left sacro-spinalis muscle. Muscle degeneration is very extensive and with edema of the stroma.

(B) One c.c. of a 1-10,000 sterile solution of adrenalin injected into the right sacro-spinalis muscle. Muscle degeneration a little more marked and extensive than the opposite side.

It was expected when this work was undertaken that insulin would be found to have a marked local effect on subcutaneous tissue and in muscle because of the edematous condition so often observed in animals treated with insulin and sugar.[1] As the foregoing observations show it was indeed found that intra-muscular injections called forth near the site of injection a very

marked degeneration of the muscular fibres with leucocytic invasion. This same type of lesion, however, was found to be caused by other agencies such, for example, as hypertonic glucose solution. Isotonic saline, on the other hand, as a rule produced only slight localized areas of degeneration although hypertonic solutions had an effect like that of insulin.

The accounts of the lesions indicated by these studies following intramuscular injections explain probably the considerable pain and discomfort that is not uncommonly complained of and it may be pointed out that if the lesions can be produced by hypertonic saline it is altogether likely that they might become quite extensive when various therapeutic agents are employed.

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THE EFFECT OF PHENOL IN THE ESTIMATION OF REDUCING SUGARS BY THE PICRAMIC ACID METHODS*

By Henry C. Sweany, Chicago, Ill.

A FEW years ago Lewis and Benedict[1] devised a colorimetric method for the estimation of blood sugar based upon the reduction of picric acid to picramic acid by an alkaline solution of the glucose present in blood. The method consists of laking 2 c.c. of blood in 5 c.c. of water, adding 15 c.c. of a saturated solution of picric acid and making the final volume up to 25 c.c. with water, taking 8 c.c. of this filtrate in a test-tube, adding 2 c.c. of a saturated solution of picric acid and 1 c.c. of 10 per cent sodium carbonate and evaporating the mixture to dryness over a free flame. The dry crystals are dissolved in 3 c.c. of water with heat, made up to 10 c.c., filtered and read in a colorimeter against a standard solution of picramic acid. The original method with various modifications has been very generally accepted among clinicians and medical research workers as it is relatively simple, rapid and presumably accurate, if properly controlled. Other factors being ignored, the accuracy depends, of course, upon the most exact adherence to the empirical details of the procedure. The most important factors are, (a) the H-ion concentration at the time of precipitation of the proteins by the picric acid, (b) the concentration of the acid remaining in the filtrate, (c) the OH-ion concentration at the time of heating, (d) the period of heating, and (e) the temperature of heating. The only variable should be the glucose concentration. All other factors should be carefully controlled and values for the various concentrations of glucose carefully determined, as in the various copper sulphate reduc-

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tion methods. Some of these factors have been quite satisfactorily controlled by various workers,[2, 3, 4] and are taken into account in Benedict's latest modification of the Lewis-Benedict method.

The method has been criticized[2, 3, 4, 5] in that the reduction of picric acid to picramic acid may also be brought about by creatinine, uric acid, polyphenols, glycuronic acid, aldehydes, ketones, etc. However, the greatest portion of these reducing substances is probably eliminated by the use of proper concentration of picric acid or the proper dilution of the filtrate. Still it seems reasonable to suspect that at least traces of these substances remain and that a summation of effects of all these reducing substances produce a marked error even in normal subjects. Any reagent that is as easily reduced as alkaline picrate must be rigidly controlled. Therefore, if the method is to be reliable in the determination of sugar in such a complex mixture as blood, especially in pathologic specimens in which unknown reducing agents may be present, it should be thoroughly tested against other standard methods or by some other acceptable means. This, it seems, has not been done satisfactorily.

Aside from the direct effect of these reducing substances, the author has noticed a combined effect produced by certain substances; the most notable substance producing this effect and the only one studied to any extent is phenol, although the polyphenols, cresols, conjugated phenols, ether, and various other substances have been found to cause apparently similar interference. For example, a concentration of phenol of about 1 part in 4000 or 2 mg. in 8 c.c. of blood filtrate will cause a trebling of the color reading, although phenol alone will cause no reduction of picric acid, neither will it cause any change in color when added *after the full color has been developed.* It must be present while the glucose molecule is in a stage of active disintegration. This effect of phenol and allied substances may be responsible for many of the discrepancies found in these methods. While the results appear not abnormal in nonpathologic specimens it is possible that the high results of blood sugar found in certain hospital patients by Myers[2] and the increase above the results obtained by the Folin method by Host and Hatlehoel[6] and Wesselow[7] may be due to a similar interference.

There are, therefore, two basic faults with the methods in question: first, the direct effect of picrate reducing substances other than glucose in the blood and second, the combined effect of substances like phenol which produce excess color in the presence of glucose. In the absence of interfering substances the question of time and temperature is not so important if a standard is *run exactly parallel to the test.* In that way the same oxidation and reduction is taking place in both mixtures and a comparative result though incomplete in both will be fairly accurate. In the presence of interfering substances, however, there is no correspondence between the standard and the unknown as we have determined by experiment. Therefore, it is not accurate to compare a blood mixture filtrate with an aqueous solution of glucose. To use an empirical color standard or even a standard picramic acid solution will run the risk of making grave errors, because the variation

of conditions from day to day varies the oxidation process of the glucose to a marked degree.

True, some of these criticisms may be applied to all blood sugar methods. It was with the idea in view to work out a rational system of comparison of the various methods and to determine if possible the extent to which phenols and other similar substances interfere with the tests that prompted this work.

The reagents used were the purest possible to secure. The picric acid was purified according to Folin and Doisy's method;[8] the glucose was the snow white anhydrous product of the Specials Chemical Co., and the other reagents were equally as well chosen. Impure picric acid causes an abnormal increase in color, a fact observed by Rhode,[9] and others. Undoubtedly the presence of traces of unchanged phenol, mono-, or dinitro-phenol, is responsible for this additional color.

Before making any tests the methods were checked with known concentrations of glucose for the various effects that might be produced by changes in concentration of alkali and picric acid; by changes in time of heating; and by changes in volume of the heated mixture. In general, both methods were sufficiently accurate in all these details to preclude any error in aqueous solutions (except the usual experimental error) when the technic outlined above was followed correctly. The results of these experiments will not be repeated here. Addis and Shevky[10] have covered these points thoroughly. Suffice it to say that sugar could be recovered within 2 to 3 per cent in aqueous solutions.

EXPERIMENTAL

In order to establish any defect in these various methods it was necessary to carry out a series of experiments: first, in aqueous solutions in which all factors could be controlled; and second, in blood mixtures in which various unknown reducing agents might be present. The Lewis and Benedict[1] method and Benedict's modification[4] were used to represent the picramic acid methods while the Shaffer-Bertrand[11] method was used as a check. This method was not used as an absolute check so much as a comparative method.

The technic of the new Benedict method is in brief as follows: 2 c.c. of whole blood are laked with 2 c.c. of water and made up to 25 c.c. gradually and with gentle rotation with sodium picrate solution. This is filtered and to 8 c.c. of the filtrate is added 1 c.c. of 20 per cent sodium carbonate solution. After mixing, this is heated for ten minutes in a water bath at 100° C. and read against a standard similarly prepared. The sodium picrate for this method is prepared by dissolving 36 gm. of purified picric acid in water, with the aid of exactly 16 gm. of sodium hydroxide, the solution is filtered, cooled and made up to 1000 c.c. It should have an acidity of 0.05 to 0.04 normal.

Shaffer's Method of Blood Sugar Analysis is as follows:

Draw 5 c.c. of blood in 25 c.c. water, heat in beaker or flask just to boiling, rotating flask gently. Add a few drops of dilute acetic acid to the hot liquid to produce visible coagulation. Five c.c. colloidal iron solution (Merck's Dialyzed) is added, the mixture is well shaken; after which about 0.2 gm.

of powdered Na_2SO_4 is added and the mixture again shaken for 10 seconds. The liquid is then poured into a 50 c.c. centrifuge tube and centrifugated for 2 minutes. The liquid is then poured off through a small filter and 21 c.c. of the water clear liquid equivalent to 3 c.c. of blood are measured into another 50 c.c. tube. Twenty-one c.c. of mixed Fehling's is added and the tube immersed in boiling water for ten minutes when the contents is again centrifugated, washed and the Cu_2O is determined by Bertrand's titration.

After working with the methods for some time, it was found expedient to adopt a more rigid technic than is called for by any of the described methods before consistent results could be obtained. For example, the blood

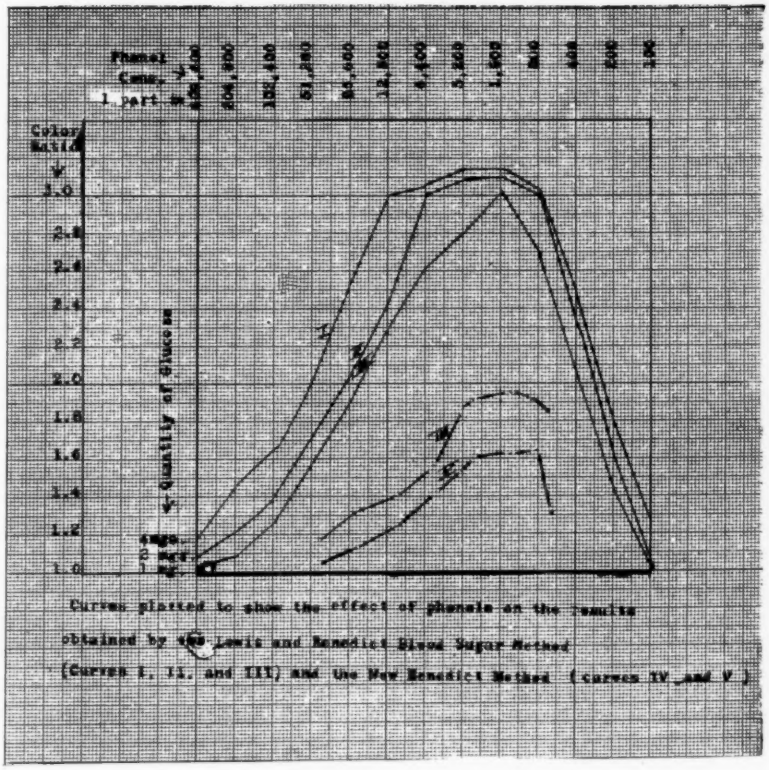

Chart 1.

was laked and precipitated in approximately two hours after drawing by exactly the same procedure each time; the precipitate was collected upon a filter within 30 minutes of the time of precipitation, and the filtrate was heated and analyzed on the same day. The tubes were well shaken before placing in the bath and the reagents were added in the same order each time.

After a cursory trial of various substances that might cause an intensification of color, it was found that most of the phenols as well as a large number of other compounds such as uric acid, creatinine, etc., gave an exaggerated reading. Phenol and potassium phenyl sulphate were then selected for a more detailed study.

A series of experiments was performed in which the phenol concentration was varied with 0.64, 1, 2, and 5 milligrams of glucose respectively. The final volume, the concentration of alkali and the picric acid, the period and temperature of heating were kept constant. The results obtained by the old Lewis and Benedict method are given in Table I and plotted in Chart 1.

TABLE I

PHENOL CONCENTRATION ONE PART IN—		409600	204800	102400	51200	25600	12800
Color Ratio	(1mg. dextrose	1.02	1.08	1.26	1.57	1.90	2.28
	(2mg. dextrose	1.08	1.21	1.37	1.73	2.04	2.43
	(4mg. dextrose	1.18	1.45	1.65	2.01	2.53	2.98
continued—	6400	3200	1600	800	400	200	100
	2.60	2.80	3.02	2.70	2.07	1.44	1.02
	3.00	3.08	3.10	3.00	2.31	1.61	1.04
	3.04	3.13	3.14	3.02	2.48	1.82	1.28

Chart 1 shows clearly the effect of phenol on the color formation. At about one part in 4000 of phenol the color is three-fold. This was repeated by the new Benedict method with one concentration of sugar, viz., 0.64 mg. in 10 c.c. of solution. The results are shown in Table II and Curve IV on Chart 1.

TABLE II

	0.64 MG. DEXTROSE				
PHENOL CONCENTRATION ONE PART IN—	40000	20000	10000	5000	3333
Color Ratio (Phenol	1.19	1.31	1.35	1.56	1.87
Color Ratio (Potassium Phenyl Sulphate	1.05	1.12	1.24	1.42	1.52

continued—	2000	1333	1000	750
	1.91	1.95	1.91	1.85
	1.60	1.62	1.64	1.30

Table II and Curve V on Chart 1 represent the results obtained with varying concentrations of potassium phenyl sulphate on 0.64 mg. glucose. The color change in this experiment reached only twice the normal instead of three times, as in the original method. By increasing the alkali as the phenol increases, the color change can be kept from decreasing after it has reached its maximum. The decrease in OH-ion concentration, therefore, accounts for the drop in the curves on the right side of the Chart.

In order to study the effect that such agents as phenol might have on these methods when applied to blood, experiments were accordingly performed on rabbits and dogs. Thus, a rabbit was bled 20 c.c. from the heart, then injected with 10 c.c. of a 2.5 per cent phenol solution and again bled 20 c.c. within 5 minutes. The two samples were analyzed for blood sugar

by the new Benedict method and by Shaffer's method. The results are found in Table III.

TABLE III

	BEFORE	AFTER
Lewis and Benedict	125	465
Shaffer	116	220

These results show a decided "false" reading in the Benedict method but also show a doubling of the value found in the Shaffer method. This could be due to the effect of the drug on the glycogenetic ferment of the liver, to "fear hyperglycemia," or some allied process. However, it is impossible by such explanations to account for the great variation between the two methods in the sample of blood taken after the injection. This we assume is caused by some interfering substance such as the phenol that was injected. To attempt to eliminate this actual hyperglycemia and to establish more clearly the effect of an interfering substance such as phenol, the work was repeated on a dog, because by proper manipulation the dog is not so susceptible to "fear hyperglycemia," and 20 c.c. of 10 per cent potassium phenyl sulphate was substituted for the phenol, because phenol may set up an irritation that will liberate blood sugar. The results are tabulated in Table IV.

TABLE IV

	BENEDICT'S METHOD	SHAFFER'S METHOD
Before Injection	0.080 mg. sugar	.069 mg. sugar
After Injection	0.110 mg. sugar	.072 mg. sugar
Difference	0.030 mg. sugar	.003 mg. sugar

This shows very little change in the results before and after injection in the Shaffer method but a definite increase by the Benedict method. This experiment demonstrates clearly the unfavorable effect of such substances with the Benedict method. In a subsequent paper a more complete comparison will be made by analyzing a large number of normal and pathologic samples of blood, using the various blood sugar methods.

DISCUSSION

The exact mechanism of this reaction is rather difficult to understand. Phenol itself will not produce any color change with alkaline picrate, nor will it modify the color if added *after* the full color has been developed in the alkaline picrate by the glucose only present when the glucose molecule is undergoing disintegration does it affect the results. The interference of phenol might be accounted for in the following manner: first, the glucose and phenol may unite, and cause increased reduction of picric acid. However, should they unite in the normal manner for phenols and aldehydes, the aldehyde group would be removed and thus there should be a lessening in the amount of color; second, the glucose-phenol combination may unite with picric acid to form a more highly colored addition compound, but this seems

unlikely; third, the phenol may react with the decomposition products of glucose to give a colored product or one which reduces the picric acid to picramic acid. The latter seems to be the most plausible explanation. Nef[12] has shown that under different conditions different quantities of end products, as well as different substances of glucose oxidation will result. Gaud[13] also shows that varying the alkali will cause a vast difference in the products of glucose oxidation. In any event, the process is not so fixed and stable as is commonly thought. It seems reasonable, therefore, to suppose that the phenol enters into the reaction in a manner that may cause a more complete oxidation of glucose fragments or may be itself oxidized to a substance like pyrochtechin (meta-dioxy-phenol) which in turn reduces more picric acid.

From a practical standpoint, however, the most important fact to consider is the true worth of these methods clinically. Especially is this true in pathologic specimens in which a true result is imperative to sound diagnosis and treatment. There is no reason to believe that phenol is the only substance that can interfere with the reaction. In fact there are good reasons to believe that there are many substances that will have a similar effect.

SUMMARY

1. It has been shown that the presence of phenol interferes with the determination of sugar by the Lewis and Benedict and the new Benedict methods, giving a two- to three-fold color when one part of phenol is present in 4000 parts of solution of phenol.

2. In the determination of blood sugar, the phenols present in normal blood cause a 10 per cent error by the Lewis and Benedict method, but practically a negligible error by the new Benedict method.

3. For a similar reason where picramic acid methods are applied to the determination of sugar in the urine without the elimination of phenols, polyphenols, etc., there will result an error of 20-50 per cent.

4. No picramic acid method can be used for the determination of sugar in solutions preserved by phenols and many related coal-tar products.

5. A possible explanation of the phenol interference has been suggested.

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CLINICAL AND LABORATORY PROCEDURES IN PEDIATRICS

By A. Levinson, M.D., Chicago, Ill.

Urine

(*Continued from page 537.*)

COLLECTION OF URINE.—From children over two years of age, urine can be obtained by having the child urinate into a vessel. From male infants urine may be collected by attaching a wide-mouthed bottle or test tube or a portion of a rubber glove over the genital organ, fastening it in place by means of adhesive plaster attached to the pubic area (Fig. 20).

From female infants, urine may be obtained by attaching a short wide-mouthed bottle over the vulva, fastening it in place by means of adhesive plaster pasted to the pubic area. Bird seed vessels and folded glass ink stands may also be used.

Catheterization.—Catheterization should be avoided as much as possible, as it traumatizes the genitals and may cause infection of the genitourinary tract. When no specimen of urine can be obtained any other way, or when a sterile specimen is desired for bacteriologic examination catheterization has to be employed.

Technic of Catheterization.—The infant is placed in the recumbent posture and the legs are spread apart. The genital organs are washed with cotton saturated in 1 per cent lysol or in alcohol, or in 1 to 5,000 bichloride of mercury solution. A sterilized metal catheter, a rubber catheter (10 French) or a glass catheter, the latter being the least desirable, is introduced for a distance of 1½ to 1⅔ of an inch in the female infant and 1 to 1½ inches in the male infant. An eustachian tube catheter (Fig. 21) is the ideal catheter for the female infant. A receptacle is held at the distal end of the catheter for the collection of the urine (Fig. 22).

Color.—In order to obtain any information from the color of the urine in infants and children, one has to keep in mind that the color of the urine changes with age, with the type of food, and with the amount of fluid consumed.

In the newly born, the urine is concentrated and often is of brick red color, supposedly due to a uric acid infarct present in the kidneys of the newly born. All through infancy, the urine is straw-colored. It becomes concentrated and assumes a light red color in diarrhea and in febrile conditions.

In older children, the urine is also straw-colored, but may become more concentrated, after the ingestion of food. At times a brick red sediment, made up of uric acid, settles at the bottom of the container after the consummation of a large amount of meat. In all febrile conditions the urine is concentrated and may assume a dark red color.

In catarrhal jaundice, as well as in icterus neonatorum the urine is greenish red in color. In hemorrhagic nephritis the urine is dark red, and at times looks like pure blood. In tuberculosis of the kidney, in calculus of the kidney, ureters or bladder the color may also be bloody. In abscesses of the pelvis of the kidney and in pyelitis the urine may be very turbid.

Specific Gravity.—For clinical purposes, the ordinary urinometer furnishes valuable information. The specific gravity in the urine of the newborn is quite high, varying between 1.020 and 1.025. During infancy, the specific gravity varies between 1.010 and 1.018. It usually changes with the time of the day and with the intake of food. A fixed specific gravity speaks for a pathologic condition. A persistently high specific gravity speaks for diabetes or acute nephritis. A persistently low specific gravity points to chronic nephritis. (See Mosenthal test.)

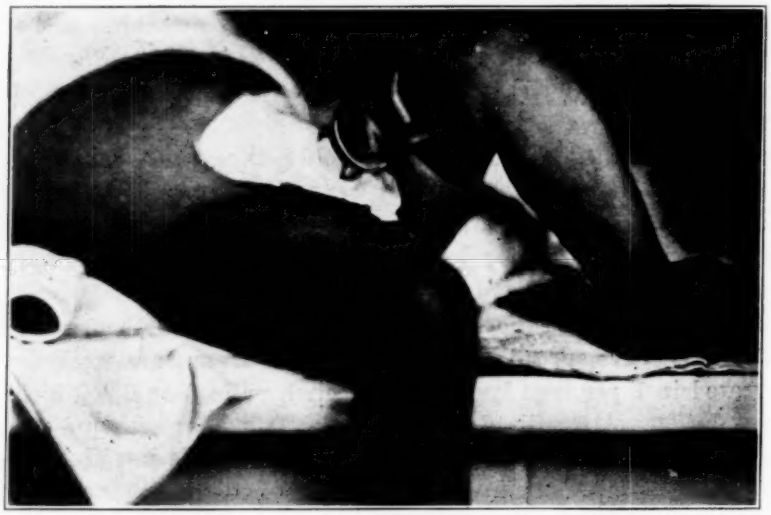

Fig. 20.—Method of collecting single specimen of urine.

Reaction.—For clinical purposes, litmus paper may be used for the determination of the reaction of urine. Immediately after the urine has been voided, it is acid in reaction. On standing several hours it assumes a neutral or alkaline reaction. Urine is often alkaline one or two hours after ingestion of food. This is known as the "alkaline tide." Neutral or alkaline reaction in a fresh specimen, not following a meal, indicates stagnation of the urine in the bladder, as happens in paralysis of the bladder.

In the administration of alkalies for the treatment of pyelitis, the reaction of urine may serve as a guide as to how much alkalies to give. As soon as the reaction of the urine becomes alkaline, the alkalies should be discontinued. Table IX from Palmer and Van Slyke may be utilized for the determination of the amount of alkali to be administered in order to turn the urine alkaline.

Albumin.—The two most simple tests for albumin in urine are: The com-

bined heat and acetic test and the nitric acid test. The combined heat and acetic acid test is done as follows:

The upper part of a tall column of urine in a test tube is heated. A few drops of 5 per cent acetic acid are added, and the urine is examined for a precipitate or turbidity by transmitted light against a dark background. A precipitate indicates the presence of albumin. Phosphates also produce a precipitate on heating, but the precipitate disappears on the addition of acetic acid, whereas an albumin coagulum will usually be intensified unless a considerable excess of acid is used.

The nitric acid test is carried out by letting a few drops of nitric acid run down from a pipette on the inner wall of a test tube containing 2 to 5 c.c. of urine. If albumin is present a white ring will appear at the contact of the nitric acid and the urine.

Fig. 21.—Eustachian tube which may be used as female urethral catheter.

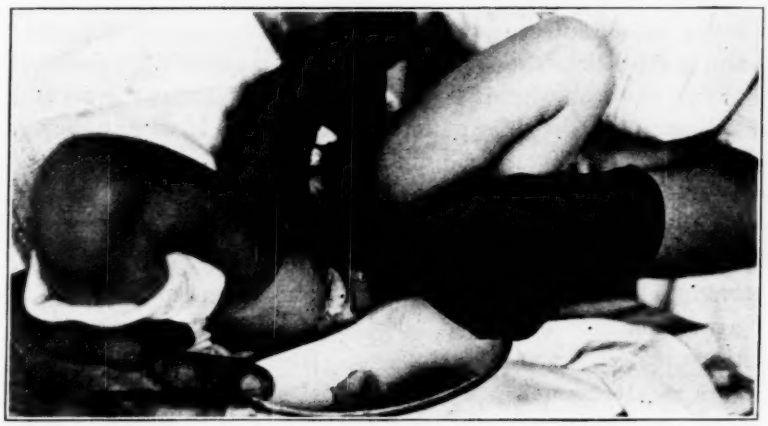

Fig. 22.—Catheterization of a female infant.

TABLE IX

SODIUM BICARBONATE PER KILO BODY WEIGHT REQUIRED TO TURN URINE ALKALINE	MINIMUM PLASMA BICARBONATE CO_2 INDICATED	MAXIMUM DEGREE OF ACIDOSIS INDICATED
gm.	vol. per cent.	
0.0–0.5	55	None
0.5–0.8	55–40	Mild
9.8–1.1	40–30	Moderate to severe
Over 1.1	Below 30	Severe

When several specimens of urine are to be examined, the nitric acid test can be simplified still further by having 5 to 6 c.c. of nitric acid in a test tube and by drawing up a small quantity of urine with a glass tube by capillary traction and introducing the lower end of the tube into the nitric acid. If

albumin is present, a ring will form in the small tube on contact of the urine and acid.

Quantitative Albumin.—An Esbach tube used for this purpose is filled with urine to the mark "U" and the reagent is added to the mark "R." The reagent used is either that of Esbach, or that of Tsuchiya.

The Esbach reagent consists of a solution of 10 grams of picric acid, and 20 grams of citric acid in 1 liter of boiling water cooled off before using.

The Tsuchiya reagent consists of 1.5 gm. of phosphotungstic acid, 5 c.c. of concentrated hydrochloric acid and 95 per cent alcohol to make up to 100 c.c.

The Esbach tube is corked and inverted several times to insure thorough mixing, and allowed to stand in a vertical position for 24 hours. The height of precipitate which settles to the bottom is read on the scale marked on the tube. The figure obtained gives the quantity of albumin in grams per liter of urine. From this the amount of albumin in the given 24 hour specimen is readily calculated. If the urine shows a heavy precipitate by the qualitative albumin test, it should be diluted 1 to 10 before the quantitative albumin reagent is added, and the final reading multiplied by 10.

Interpretation.—A negative albumin test is valuable in excluding nephritis. A positive albumin test does not necessarily mean the presence of a nephritis, as all infectious diseases may show a temporary albuminuria. There is also a condition known as orthostatic albuminuria, where albumin is found in the urine of nonnephritic patients when the patient is up and around, and which disappears when the patient is in bed. It is wise, however, to keep all patients showing albuminuria under observation, so as to not overlook a nephritis. Qantitative albumin determination throws light on the amount of destruction going on in the kidneys, or the amount of protein of the blood permeated through the kidneys.

Qualitative Determination of Sugar.—Haines', Fehling's and Benedict's methods are commonly employed. Fehling's method has the disadvantage of consisting of two separate solutions. Haines' and Benedict's reagents are therefore most commonly used. Haines' solution is made by dissolving 8.314 gms. of copper sulphate in 400 c.c. of water, adding 40 c.c. of glycerin, and then 500 c.c. of 5 per cent potassium hydroxide. Two to 3 c.c. of Haines' solution is placed in a test tube and boiled gently. No precipitate should form. Several drops of urine are now added and the mixture brought to a boil. A yellow-red precipitate of copper oxide forms immediately or in a few minutes if sugar is present in the urine.

Qualitative Determination of Sugar.—Haines', Fehling's and Benedict's methods may be used, the Benedict method being most frequently used.

The Benedict reagent is prepared as follows:

One hundred grams of anhydrous sodium carbonate, 200 gm. of sodium citrate. and 125 gm. of potassium sulphocyanate are dissolved over a flame in about 800 c.c. of water and filtered. In another vessel is dissolved 18 gm. of crystallized copper sulphate in 100 c.c. of water. The copper sulphate solution is now slowly added to the filtrate with constant stirring.

Twenty-five c.c. of this reagent will reduce 0.050 gm. glucose. It may be

necessary to titrate the Benedict's solution to make certain that it will reduce 0.050 gm. of glucose. For the examination of the urine the following procedure is employed: Twenty-five c.c. of the Benedict reagent is measured into a porcelain evaporating dish, 5 or 10 gms. of solid anhydrous sodium carbonate is added. A small amount of talcum powder may be added to prevent bumping. This is now heated over a flame until the carbonate is dissolved. From a burette, urine, diluted 1 to 10, is run in until the blue color disappears, this being the end point. The reagent should be kept boiling during the titration.

To obtain the percentage of sugar in the urine, the following formula is used: 0.050 (amount of glucose reduced by quantity of reagent taken) is divided by X (the number of c.c. of diluted urine taken), times 1000 c.c. (100 x 10 which was the dilution of urine) or $\frac{0.050}{X \times 1000}$

Interpretation.—The reduction tests for sugar are at times misleading. Often a reduction is obtained with Haines' or Benedict's solution that is grayish in color. This is due to the ingestion of salicylates or acetylsalicylic acid and must not be taken to indicate the presence of sugar in the urine. When in doubt, the fermentation test or the phenylhydrazine test should be employed. Ingestion of large quantities of sugar will give a temporary positive sugar test. When sugar is present in urine, the diagnosis of diabetes should not be made until several specimens have been examined and until the food factor (ingestion of large amounts of sugar) has been excluded, and until the sugar in the blood has been determined and found to be higher than normal. The latter will exclude renal diabetes.

Acetone.—To a few c.c. of urine are added a few drops of a freshly prepared solution of sodium nitroprusside and a few drops to ½ c.c. of glacial acetic acid. A small quantity of ammonium hydroxide is superimposed on it. A violet contact ring appears and becomes intensified in a minute or two if acetone is present in the urine.

Diacetic Acid.—Gerhardt's test is used. A few drops of 10 per cent ferric chloride are added to the urine. If a precipitate of phosphate forms, ferric chloride is added until no more precipitate forms, and the solution is filtered. In the presence of diacetic acid a Burgundy-red color appears. The color becomes weaker, or disappears on heating; if caused by drugs, like salicylic acid, aspirin, salol, diuretin and phenacetin, the color persists.

Acetone in urine may be due to diabetes. In children, however, it is often present in urine in cases of prolonged starvation. Too much weight must therefore not be placed on the presence of acetone, except as an indication of starvation.

Indican in the urine was at one time supposed to be an indication of the amount of putrefaction in the bowels. This, however, does not seem to be true.

Microscopic Examination.—In acute nephritis casts and cells are present in the urine in such large amounts that they may be detected under the microscope in an uncentrifuged specimen. In less severe conditions the urine should be centrifuged, the supernatant fluid poured off, and the sediment examined on a clean slide under the microscope. The sediment should be

spread in a thin layer, otherwise the examination is unsuccessful. A cover glass over the slide helps to even the layer of sediment.

CASTS.—The presence of casts in urine indicates a destructive process going on in the kidneys. Granular and hyaline casts are most important in the diagnosis of nephritis. Epithelial casts do not justify the diagnosis of nephritis. Care must be taken to differentiate between casts, crystals and debris.

CELLS.—The presence of many red cells in the urine indicates a hemorrhage, or at least a marked irritation of the kidney. Such is the case in tuberculosis of the kidney and in hemorrhagic nephritis. An occasional white cell in the microscopic field is present even in normal urine. In pyelitis the white cells are present in large numbers, and are usually clumped.

In examination for tubercle bacilli the urine, which has preferably been standing in the refrigerator for 12 to 24 hours, is centrifuged for ½ to 1 hour and the sediment is stained by the Ziehl-Neelsen method. Enough alcohol should be used for decolorization in order to exclude smegma bacilli.

CRYSTALS.—Uric acid crystals are of no significance. Their presence does not indicate an increased uric acid content of the urine.

Urates, calcium oxalate, and calcium carbonate are of no significance.

Triple phosphates, ammonium, magnesium and calcium phosphate are of significance. They occur in alkaline urine, and usually signify stagnation. The conditions are observed in paraplegia, chronic pyelitis, and chronic cystitis.

BLOOD.—In addition to the microscopic method, a chemical method may be used. The guaiac test is most often employed. The technic is as follows:

Freshly prepared tincture of guaiac (small amount of guaiac dissolved in a few c.c. of absolute alcohol) is added to 5 c.c. of the urine until the urine becomes turbid. Hydrogen peroxide is now added, drop by drop, until a blue color is produced, or until 2 c.c. has been added. If the urine contains either blood or pus, a deep blue color results.

BILE.—A few c.c. of urine is superimposed on 1 to 2 c.c. of concentrated nitric acid. A green ring, which may change to blue, violet, red or yellow, forms at the line of contact.

QUANTITATIVE TESTS.—The following are most important quantitative tests:

Amount in 24 hours
Albumin and sugar when present (described above)
Total nitrogen
Urea nitrogen
Creatinine
Chlorides

AMOUNT.—The amount of urine excreted in 24 hours is by itself a good kidney function test. An increased amount of urine is present in diabetes insipidus, and in chronic interstitial nephritis. The urine is decreased in amount in all febrile diseases, and in cardiorenal affections. As an arbitrary standard, 12 ounces should be considered the minimum amount of urine to be voided in 24 hours by a child between 6 to 12 years of age, and 16 ounces in 24 hours by a child above 12 years of age. The intake of fluid should be

measured in conjunction with the urine output, as the latter is naturally influenced by the former.

In administering digitalis, the 24 hour urine output is an important guide in the therapy. If the urine is increased in amount after the administration of the drug, the drug is performing its function. If the urine output is not increased by the medication, the digitalis is not producing its effect on the heart, or the decreased elimination of urine may be due to renal disturbances, instead of cardiac, for digitalis has no, or only slight, diuretic effect in renal conditions.

TOTAL NITROGEN.—The total nitrogen in urine varies with the amount of protein ingested. On a mixed diet the total nitrogen output varies between 4 to 7 gm. in 24 hours, in normal children five to ten years of age. This amount is lessened in nephritis with retention, and in uremia.

Method of Determination.—The urine is diluted according to its specific gravity.

SPECIFIC GRAVITY	DILUTION
1.010 or below	20:100 or 1:5
1.010 – 1.020	15:100 or about 1:6½
1.020 or above	10:100 or 1.10

One c.c. of the diluted urine is pipetted into a Pyrex nitrogen tube, and 2 c.c. of acid digestion mixture (see section on Blood, footnote No. 4) added. A glass bead is put into the mixture to prevent bumping. The mixture is now digested over the microburner, the same as in the determination of non-protein nitrogen constitutents in the blood. After the addition of water the solution is transferred to a 200 c.c. volumetric flask. In another 200 c.c. flask a standard is made up containing 5 c.c. of the ammonium sulphate standard solution, 2 c.c. of acid digestion mixture, and about 125 c.c. of water. To each are added 30 c.c. of Nessler's solution (see section on Blood, footnote No. 6.) and water to the mark. Readings are made against the standard and calculated according to the formula:

$$\frac{\text{Standard}}{\text{Reading}} \times \text{dilution} \times \frac{1}{1000} \times \text{24 hour volume} = \text{gm. of total nitrogen in 24 hours.}$$

UREA NITROGEN.—Urea nitrogen varies normally between 3 to 5.5 grams in 24 hours, or about 80 per cent of the total nitrogen. The amount is decreased in nephritis with retention and in uremia. It also varies in small limits with the amount of protein ingested.

Method of Determination.—One c.c. of the diluted urine (see total nitrogen in urine) is measured into a Pyrex tube. One drop of buffer mixture (see section on Blood, footnote No. 7), and one c.c. of urease solution (footnote No. 8) are added. The mixture is placed in water at 55° C. and allowed to stand for ten minutes. It is then transferred to a 200 c.c. volumetric flask. The standard is now prepared, consisting of ammonium sulphate standard solution, 1 c.c. of urease solution, and about 100 c.c. of water. Twenty c.c. of Nessler's solution are added to each, and water to the mark. Readings are made against the standard and the result calculated according to the formula:

$$\frac{\text{Standard}}{\text{Reading}} \times \text{dilution} \times \frac{1}{1000} \times \text{24 hour volume} = \text{gm. of urea nitrogen in 24 hours.}$$

A less accurate method of determining urea in the 24 hour quantity of urine, but one used extensively by clinicians is the hypobromide method, which consists of filling the Doremus' ureometer with sodium hypobromide and instilling 1 c.c. of urine into the ureometer with a curved pipette. The gas replacing the solution on the top is read off by the number on the ureometer and converted to percentage. Thus a reading of 0.01 equals 1 per cent urea, etc.

CREATININE.—The amount of creatinine in children's urine varies between 0.3 to 0.5 gm. in 24 hours.

Technic.—Into a 100 c.c. volumetric flask is measured 1-3 c.c. of urine. Into a similar flask is measured 1 c.c. of standard creatinine solution containing 1 mg. of creatinine (see creatinine in blood). To each is added 20 c.c. of saturated picric acid solution, and then, noting the time, 1.5 c.c. of 10 per cent NaOH. After standing for just ten minutes, the solutions are made up to the mark with water and read in the colorimeter, the standard being placed at 20.

$$\frac{\text{Standard}}{\text{Reading}} = \text{mg. of creatinine in volume of urine taken.}$$

CHLORIDES.—The amount of chlorides in normal urine varies between 2 to 4 gm. per 24 hours or between 0.7 to 1.0 per cent. Anything below 0.7 per cent speaks for the possibility of a threatening edema. The quantitative determination of chlorides therefore becomes a valuable kidney function test.

Two solutions are prepared for the test. Solution 1 consists of the following:

Anhydrous, crystallized silver nitrate, (C. P.)	29.055	gm.
25% Nitric acid in distilled water	900.	c.c.
Cold saturated solution of ammonioferric alum in distilled water	50.	c.c.
Distilled water, q. s.	1000	c.c.

Solution 2 consists of:

Ammonium sulphocyanate	7	gm.
Distilled water, q. s.	1000	c.c.

Solution 2 is intentionally made too strong, and must be standardized by adding distilled water in such an amount that exactly the last drop of 2 c.c. of this solution will bring about the end reaction when added to 1 c.c. of Solution 1. The end reaction consists of a reddish brown color, which does not disappear on moderate stirring. If the second last drop produces a discoloration which disappears rather slowly, and the last drop a deep brown color, the solution must be still further diluted, until the discoloration on the addition of the last drop is a light reddish-brown, which does not disappear on stirring fifteen to twenty seconds.

The test proper is made as follows: 0.5 c.c. of urine to be tested is placed in a porcelain dish, 1 c.c. of Solution 1 is then added and the mixture is stirred for about a minute with a glass rod. Solution 2 is now added drop

by drop by means of a 2 c.c. pipette graduated to at least .05 c.c. and the mixture is stirred until the brown color developing after each drop disappears. The amount of Solution 2 which has been used to bring about the end reaction is now read off, and the difference between this and 2 is equal

TABLE X

CONSTITUENTS OF URINE IN NORMAL CHILDREN

Amount in 24 hours	360 to 480 c.c.
Specific gravity	1010 to 1018
Albumin	Negative
Sugar	"
Acetone	"
Casts	"
Cells	Few
Chlorides	0.7–1.0%
Chlorides (in 24 hours)	2 to 4 gm.
Total Nitrogen (in 24 hours)	4.0 to 7.2 gm.
Urea Nitrogen (in 24 hours)	3 to 5.5 gm. (80% of total nitrogen)
Creatinine (in 24 hours)	0.3 to 0.5 gm.
Ammonia Nitrogen	0.7 to 1.2%

to the number of grams of sodium chloride per 100 c.c. of the specimen tested. If, for example, it takes 1.26 c.c. of Solution 2 to bring about the end reaction, the amount of chloride in 100 c.c. of urine equals 2.-1.26 which equals 0.74 per cent of chlorides.

TABLE XI

CHEMICAL CONSTITUENTS OF BLOOD AND URINE IN SAME CHILD (NORMAL)

BLOOD

Mg. per 100 c.c.

Nonprotein Nitrogen	Urea Nitrogen	Uric Acid	Creatinine	Chlorides	Sugar
26.1	12.6	3.2	1.1	5.75	100

URINE

c.c. per 24 hours

Quantity in 24 hours	Total Nitrogen	Urea Nitrogen	Creatine	Creatinine	Chlorides
540	4.56	4.10	0.037	0.34	3.93

KIDNEY FUNCTION TESTS.—The two most commonly used tests are: The phenolsulphonephthalein and the Mosenthal.

PHENOLSULPHONEPHTHALEIN TEST.—A sterile solution of phenolsulphonephthalein, containing 6 mg. of the dye per 1 c.c. (may be obtained in ampoules) is injected subcutaneously. The patient is given all the water he wants during the test so as to cause diuresis. Ordinarily, the dye will appear in the urine in 10 minutes. Urine is collected during the first, second, and third hour in separate bottles.

Each specimen of the collected urine is made alkaline with 25 per cent sodium hydrate, and diluted to 1 liter, or less, according to the amount of urine, and the readings are made by comparison with standard color tubes obtained on the market, or by means of a Hellige colorimeter. Blood. if

present, may be removed by using powdered lime or milk of lime, instead of sodium hydrate. This makes the solution alkaline and precipitates the blood pigments.

In normal children 70 per cent to 80 per cent of the injected phenolsulphonephthalein is excreted in 2 hours. Forty to 50 per cent the first hour, and 20 to 30 per cent the second hour. In nephritis, the excretion of the dye is usually lowered according to the damage to the kidneys.

The phenolsulphonephthalein test, I believe, is only corroborative, but no diagnosis or prognosis should be based on it. I have seen patients in whom the phenolsulphonephthalein excretion was down to 5 per cent, who recovered, and others with 80 per cent phenolsulphonephthalein excretion, who died of insufficient kidney function.

MOSENTHAL TEST.—The test lasts 24 hours. The bladder is emptied at 8 A. M. and the urine discarded. The patient is given his customary meal, and in addition, 1 quart of fluid. The same is done at noon, and at 5 P. M. No food is allowed at any other time. The amount of fluid may be increased or decreased according to the weather and to the age of the child.

The urine is collected in 2 hour specimens until 8 P.M., the patient voiding at the end of each 2 hour period in order to make each specimen complete. The night urine, from 8 P.M. to 8 A.M., is collected as one specimen.

The volume and specific gravity of each specimen is measured. When there is a disturbance in kidney function, the quantity of night urine increases above normal and shows a low specific gravity. In advanced functional disturbances of the kidneys, the specific gravity becomes fixed and low.

REPORT OF A CASE OF ERYTHREMIA*

By Irwin S. Sutton, M.D., and William Cole, M.D., Anaheim, Calif.

CASE No. 12753.—Mr. M. S. W., a widower, aged 49, complained of soreness and swelling of the left knee.

Family History.—His father died at 50 of a tropical fever and his mother at 51 from tuberculosis. He has three brothers all of whom are healthy adults. His wife died, aged 51, of consumption and had never been pregnant.

Past History.—He had never had malaria, tuberculosis, or syphilis, but in 1914 had had a severe attack of typhoid fever. The same year he received a severe blow to the left patella which he believes was fractured. After rest in bed for several months he made a complete recovery. While in the hospital in 1921 because of a crushing injury of the left foot he developed a collection of fluid in the scrotal sac without apparent reason, and which disappeared without any treatment. Lately he has noted moderately severe temporal throbbing headaches which are gradually increasing in intensity.

Present History.—In November, 1922, he suddenly developed a large amount of fluid about the left knee joint which was tender on motion. The swelling increased under the ministrations of a chiropractor and has lately interfered with sleep. Recently he has had several attacks of epistaxis but no subcutaneous hemorrhages. There have been a few fleeting joint pains and some embarrassment of the heart on exertion. He has lived for twenty years in this locality where the altitude is low. Since October, 1922, he has had nocturnal and diurnal frequency of urination. Several of his friends have recently complimented him upon his ruddy complexion.

Physical examination.—The patient was a middle-aged, poorly nourished, stoop shouldered man, markedly erythrotic. The conjunctivæ were extremely injected, and the face, neck and hands were of a dark reddish hue, changing to a dusky purple on exposure to a draught of cold air. The tongue and oral mucosa were of an intense scarlet tint. The hair and nails were rather dry and lusterless and there was a pronounced tendency to ichthyosis. Pupils were concentric, equal and active. Ocular movements were normal. Ophthalmoscopic examination revealed engorgement of the retinal vessels and moderate edema of the nerve heads.

The nasal examination.—The nasal mucosa and turbinates were congested. There was a superficial ulceration of the septum on the left. The gums were spongy and bled easily on pressure. Roentgenograms revealed two infected teeth. There was a slight general adenopathy. There was no demonstrable thyroid enlargement.

Chest examination.—There was some flattening of the left side anteriorly and a marked depression at the xiphoid. There was also some general

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From the Johnston-Wickett Clinic, Anaheim, Calif.

emaciation. The breath sounds were rather harsh throughout and some crackles were heard over the splenic area which suggested a perisplenitis. Stereo-roentgenograms revealed a normal heart shadow and a few fibrous bands in the upper part of both lungs. The cardiac examination was essentially negative but the blood pressure was rather high (systolic 170, diastolic 110).

Abdominal examination.—The skin was rather pale, contrasting with the general erythrocytic appearance. The abdomen was rather tympanitic and prominent below the umbilicus. Liver dullness extended from the fifth rib in the nipple line to the costal margin. It was impossible to palpate the spleen. The external genitalia were negative and the prostate was not enlarged.

Orthopedic examination.—The general carriage was slouchy, with some kyphos and bowing of the legs. The left knee joint was tense from a collection of synovial fluid. There was no muscle spasm, local heat, or redness. A roentgenogram showed a slight roughening of the articular surfaces. Aspirated fluid was sterile. With rest in bed and a pressure bandage the fluid rapidly disappeared. There was tibial periostitis. Neurologic examination was negative except for a weak to absent right ankle jerk.

A twenty-four hour urine specimen contained a strong trace of albumin, specific gravity was normal and the specimen was highly acid. The serum Wassermann reaction (two antigens) negative. Basal metabolism, average of the readings was +42.7. A glucose tolerance test showed a normal curve.

Blood examination.—Blood sugar 0.17, creatinine 1.8 mg., uric acid 2.7 mg., nonprotein nitrogen 52.4 mg., chlorides 0.6 per cent. CO_2 tension (Van Slyke) 68.3 volumes per cent. Red cell fragility test—Hemolysis began at 0.48 per cent and was complete at 0.28 per cent. The clotting time varied from two to four minutes and stained and unstained blood smears showed normal erythrocytes.

The hemoglobin (Dare) was estimated at 202 per cent and erythrocyte count was 10,760,000. This was checked with a U. S. certified pipette and found accurate. The color index was 0.9445. The leucocyte count was 9,200, with 76 per cent polynuclears; 19 per cent small mononuclears, 3 per cent large mononuclears and 2 per cent transitional cells.

Discussion.—This patient complained of a painful swollen knee and was unaware of any blood changes. His color suggested heart disease but this organ was practically normal. The low blood sugar and the negative urine eliminated diabetes as a cause of the polycythemia. The fact that his wife and mother died of pulmonary disease would make it rather difficult to eliminate this factor. He has lived for some time at a mean altitude of thirty feet above sea level and has not had malaria. The joint lesion disappeared under pressure bandages and did not at any time suggest tuberculosis. The fluid accumulation was possibly due to a change in blood volume, associated with some trauma of the knee. A review of the literature shows the blood count in this case remarkably high and that it is the only case reported with joint symptoms.

A COMPARATIVE STUDY OF VARIOUS METHODS OF HEMOGLOBIN DETERMINATIONS*

By Elmer G. Senty, M.D., Rochester, Minn.

INTRODUCTION

HEMOGLOBIN is the oxygen-carrying constituent of the blood. It is a complex proteid, which, on decomposition, breaks up into globulin (96 per cent) and a simpler pigment, hematin (4 per cent[10]). The iron content is about 0.334 per cent, or 1 mg. for each 300 mg. of hemoglobin. The estimation of hemoglobin is a very important procedure in clinical diagnosis; it is of value in determining whether patients with pallor are really anemic. Then, too, the hemoglobin content of the blood is a necessary factor in determination of the color index in diseases such as pernicious anemia and chlorosis. The normal hemoglobin in adults is 13 to 14 gm. for each 100 c.c. of blood (100 per cent). The hemoglobin in infants during the first few days of life is very high, usually about 130 to 135 per cent; in young children 75 to 80 per cent; in adults 95 to 100 per cent, while the percentage is usually lower than 100 per cent after the age of sixty-five years. Haldane and Smith assert that the depth of color in the blood is directly proportional to the oxygen capacity, the percentage of hemoglobin present in other words. In the estimation of hemoglobin, various methods have been employed, aiming at accuracy and ease of determination.

HISTORY OF THE DEVELOPMENT OF EXISTING METHODS

In 1878, Gowers introduced his hemoglobinometer. This instrument consists of two tubes about 11 by 0.8 cm. One tube, closed at both ends, contains the standard solution, 2 c.c. of gelatine tinted with picrocarmin. The color of this standard corresponds as nearly as possible to a 1 per cent hemoglobin solution. In the other tube the level attained by 2 c.c. of fluid will represent 100 per cent of hemoglobin. This tube is graduated to read hemoglobin percentage from 10 to 140 per cent. In making a hemoglobin estimation, 0.20 c.c. of blood is obtained in a graduated pipette and blown into the graduated tube which contains a little water. This is thoroughly mixed and the pipette washed by frequently drawing the solution into the pipette. Water is added, drop by drop, and the solution intimately mixed, until the colors in both tubes are similar. The percentage of hemoglobin is then read at the height of the solution in the graduated tube. This instrument is very simple, handy and inexpensive, but only fairly accurate; the picrocarmin solution fades and

*Abridgment of thesis submitted to the faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Master of Science in Medicine, October, 1922. Work done under the direction of Dr. A. H. Sanford, Section on Clinical Pathology, Mayo Clinic.

the same standard tube cannot be used with artificial light. Then, too, the standard solution is not made of blood and consequently there is some difficulty in the accurate matching of the solutions.

Fleischl,[16] in 1885, devised a new hemoglobinometer which has since been much improved by Miescher, now known as the Fleischl-Miescher instrument, consisting of a stage similar to that of a microscope. In the center is placed a cylindrical chamber divided into two equal parts and having a glass bottom. Half of the chamber is filled with water, the other half with a 1:200 dilution of blood with a 1 per cent solution of sodium carbonate. A purple-stained, glass wedge, or color prism, with the hemoglobin percentage values marked along the edge, slides beneath the chamber, and is moved from side to side by a screw. Light from a candle is reflected by a plaster-of-Paris reflector and illuminates the chamber from below. The glass wedge beneath the chamber is moved back and forth until the color of the portion of the wedge beneath the chamber containing the water is similar to that of the blood solution in the opposite chamber. The position of the wedge is read through a small window at the back of the stage, and represents the percentage of hemoglobin present. Each instrument has a scale which gives the milligrams of hemoglobin for each liter of blood according to the readings made by that particular hemoglobinometer. When the dilution of blood is known, it is possible to determine the amount of hemoglobin in grams for each 100 c.c. On account of the accuracy of this instrument, it has always been popular in experimental work. It is, however, quite cumbersome and expensive, and must be used in a dark room with a yellow light; these objections, together with the time required and the technical difficulties make it impractical for general clinical use.

In the year 1892, Hoppe-Seyler described a method of determining the percentage of hemoglobin, whereby the blood to be examined was saturated with carbon monoxide. This solution was compared with a known carbon monoxide standard solution. The method has proved too complicated for general clinical use.

Haldane and Smith, in 1898, suggested a new method of estimating the hemoglobin content of the blood. They noticed that, on the addition of potassium ferricyanide to a moderately diluted solution of blood, gas bubbles arose which were found to be pure oxygen. With this in mind, they devised an instrument for liberating and measuring the oxygen from the blood, and determined the oxygen capacity of normal blood of the adult male to be 18.5 volumes per cent, which was taken as an equivalent of 100 per cent hemoglobin; that of women 16.5 volumes per cent, and that of children 16.1 volumes per cent. The method has been much improved technically by Haldane, Bancroft, Van Slyke and others. While not practical for routine hemoglobin determination, it is nevertheless, one of the most accurate methods. It is of inestimable value in the preparation of standard hemoglobin solutions.

Tallqvist's simple and unique hemoglobinometer was introduced in 1900. This instrument consists of a booklet of prepared white filter paper and a lithographed color scale representing the hemoglobin content of blood to be

from 10 to 100 per cent. The tints of the standard are prepared with water colors, and represent the color produced by undiluted blood soaked in filter paper, the hemoglobin values having been determined by the Fleischl-Miescher instrument. A rather large drop of blood is touched to a piece of the filter paper and allowed to distribute itself over a portion of it. As soon as the stain has lost its humid gloss, and before coagulation can take place, the stained filter paper is laid beneath the circular openings of the color scale, and at the point where the colors match, the percentage of hemoglobin is read. Because the colors of the scale vary by 10 per cent, an approximate value only can be obtained, and this reading may vary if the color is matched too soon or after coagulation has begun. The method cannot be recommended for accuracy in routine clinical use.

In 1900, also, Dare devised a new instrument for hemoglobin estimation. The use of this instrument is based on the premise that the color of a thin film of undiluted blood illuminated by artificial light can be compared with

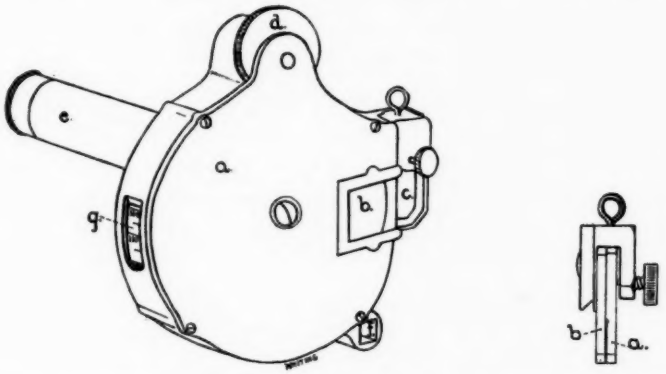

Fig. 1.—Dare hemoglobinometer and capillary pipette of Dare hemoglobinometer.

a graduated color comparison standard. This standard is a color prism made of ruby glass; the 100 per cent reading represents 13.7 gm. of hemoglobin for each 100 c.c. of blood. Hemoglobin estimations can be made from 10 to 120 per cent.

The instrument (Fig. 1) consists of a case (a) enclosing the color-prism comparison standard, with an aperture (b) in the case, admitting light to the color prism, a pipette (c) made of two thin pieces of glass, one opaque and one transparent, with a surface of definite thickness (about 0.18 mm.) between; a small screw (d) to move the color prism; telescope (e) to focus and magnify; an opening (f) for the attachment of the candle or the electric light equipment, which is part of the newer instrument. For the purpose of color comparison, blood is drawn by capillary attraction into the small thin chamber of the pipette which has a sufficient surface for color field and illumination by artificial light. Between the film of blood and the source of illumination, a white glass interposes to diffuse the direct rays of light. The shades of color are best observed against this white background. The pipette is slipped into the instrument (Fig. 1b), candle or artificial light is

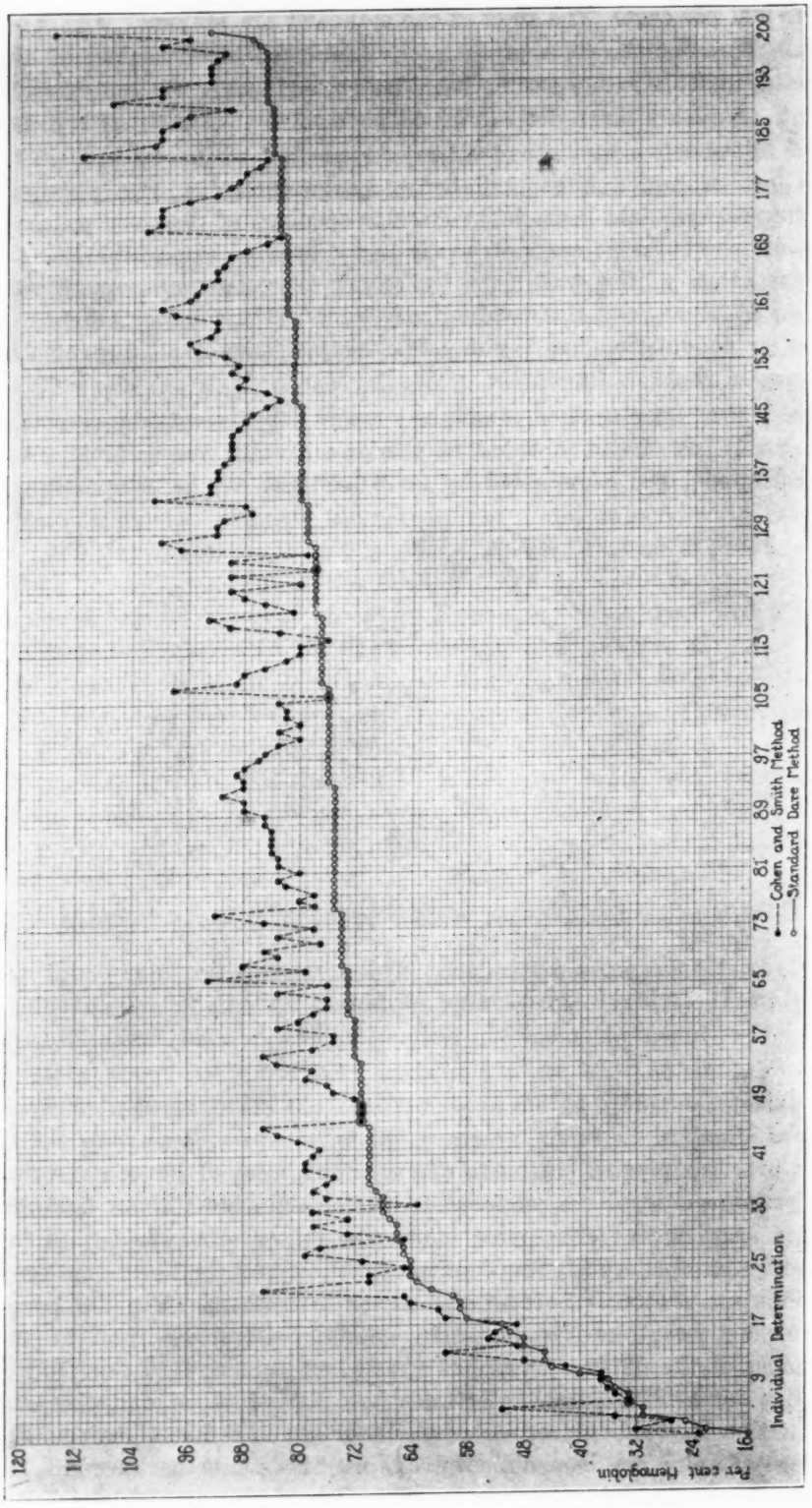

Fig. 2.—Standard Dare readings and readings by the Cohen and Smith acid hematin method. Except in isolated cases, readings by both methods check up to approximately 65 per cent. Above 65 per cent, and especially above 70 per cent, the calculations on the standard Dare as compared with those obtained by the Cohen and Smith method, show marked variations.

used, and the prism is rotated until the colors match, when the hemoglobin percentage is read through the notch (g) at the edge of the case. As suggested by Dare the advantages of his instrument are:

"1. The technic and dilution errors are eliminated. Turbidity due to leukocytosis becomes almost imperceptible with transmitted light against an opaque background.

"2. The shades of color are very decided even though the hemoglobin content is low.

"3. The time element is very short.

"4. The capillary pipettes are standardized and are consequently interchangeable."

This instrument is very simple and convenient, but quite expensive and must be handled carefully.

Haldane described a revised method of hemoglobin determination in 1901. He used the Hoppe-Seyler principle with the Gowers' hemoglobinometer and claimed very satisfactory results. The standard solution used is a 1 per cent solution of ox or sheep's blood saturated with carbon monoxide and having an oxygen capacity of 18.5 volumes per cent, which value has been quite generally accepted and represents 100 per cent hemoglobin. In using this method 20 c.c. of blood is delivered to the graduated tube, containing as much water as safely possible. Before mixing, a narrow glass tube, connected with a gas tap, is pushed down almost to the level of the liquid. The gas is turned on, displacing the air in the tube. The glass tube is then slowly withdrawn, with the gas still flowing, and the top of the graduated tube quickly closed with the finger. The solution is thoroughly mixed until the pink tint of carbon monoxide hemoglobin appears. Water is added, drop by drop, from a pipette until the tints in both tubes are similar. The percentage is then read at the height of the fluid in the graduated tube. Another drop or two is added to produce a change in color, and the mean of the readings is accepted as the correct percentage of hemoglobin present. The tints are best compared against sunlight or an opal glass light if artificial light is desired. This method is fairly accurate but, as asserted by Palmer, the standard is not permanent.

Perhaps the most simple and popular hemoglobinometer in use today is that described in 1902 by Sahli who, for the first time, used an acid hematin standard. The instrument is similar to that of Gowers except that the standard is a 1 per cent solution of acid hematin instead of a picrocarmin solution corresponding to a 1 per cent solution of blood. The blood to be tested is likewise changed into acid hematin. In determining the percentage of hemoglobin 0.20 c.c. of blood is obtained in the Sahli pipette and transferred to the graduated tube which contains 0.1 normal hydrochloric acid up to 10 per cent. The tube is well shaken, and when the clear, dark brown color due to the formation of acid hematin, appears, distilled water is slowly added and the contents carefully and thoroughly shaken until the color of the mixture corresponds to that of the standard solution. At this point the percentage of hemoglobin is read; it corresponds to the level of the solution in the grad-

uated tube. This instrument has a ground glass background which diffuses the light before it reaches the tube, thereby excluding all disturbing reflections. It can be used with artificial light and in a lighted room. The color comparisons, of course, will be more accurate if the standard and tested solutions are of similar composition. For this reason the Sahli instrument is superior to that of Gowers or to other methods using foreign standard solu-

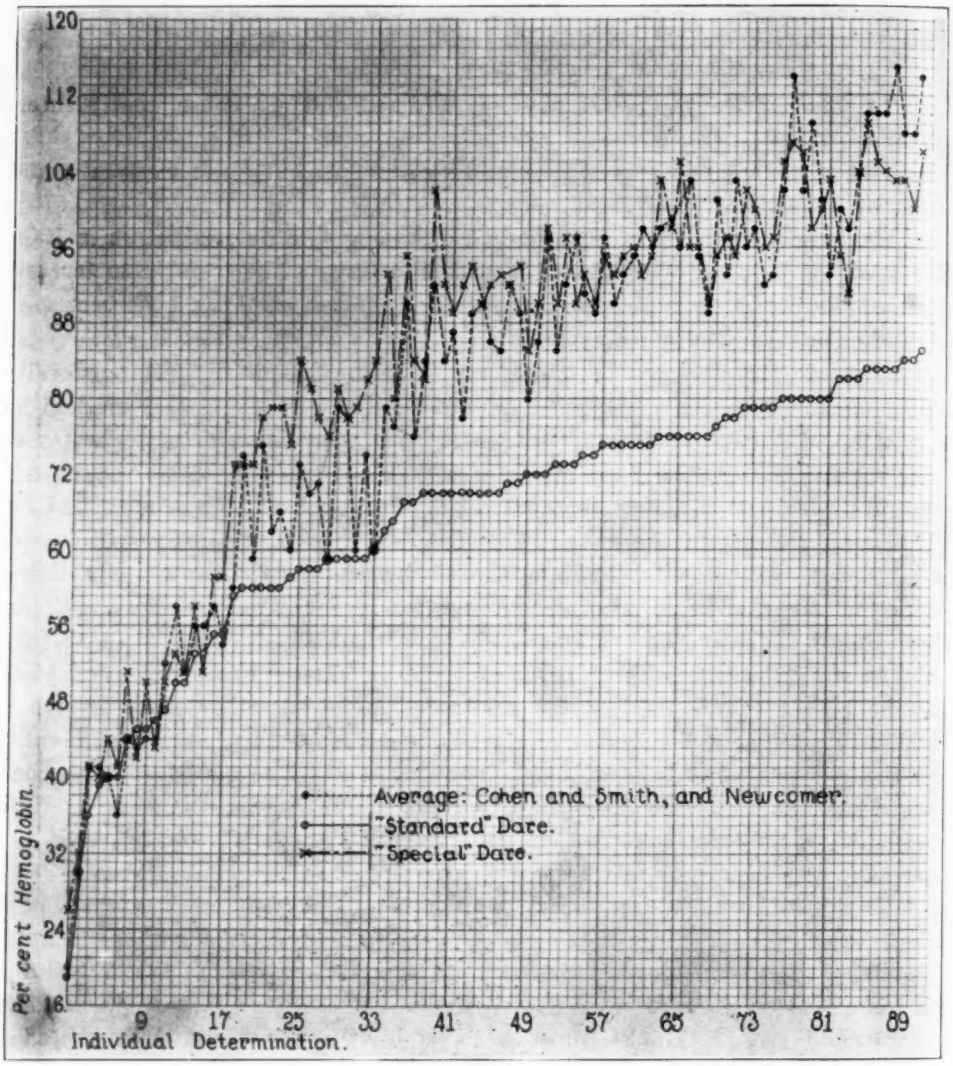

Fig. 3.—Average readings by the Newcomer and Cohen and Smith methods and readings by the standard and special Dare instruments. In this curve also, the values are similar up to approximately 60 or 65 per cent. Readings of from 60 to 80 per cent on the standard Dare follow as in Fig. 2, while the special Dare reads approximately 10 to 15 per cent higher. The readings on the special Dare, in determinations from 80 to 115 per cent, compare favorably with the average readings by the Cohen and Smith, and Newcomer methods.

tion or colored glass as standards. Sahli tubes on the market today fade so markedly as to make them unfit for use, other than comparison, unless frequently checked and restandardized. In the Sahli method of hemoglobin estimation 17.2 gm. for each 100 c.c. represents 100 per cent, and the deter-

minations must consequently be reduced if 13.4 gm. for each 100 c.c. is to be considered as standard. Robscheit says that the tubes purchased today show fading varying from 5 to 20 per cent. Jacobson has suggested a new standard for the Sahli hemoglobinometer, "rufigallic acid." He makes this solution by adding 5 to 10 drops of concentrated sulphuric acid to 100 c.c. of a

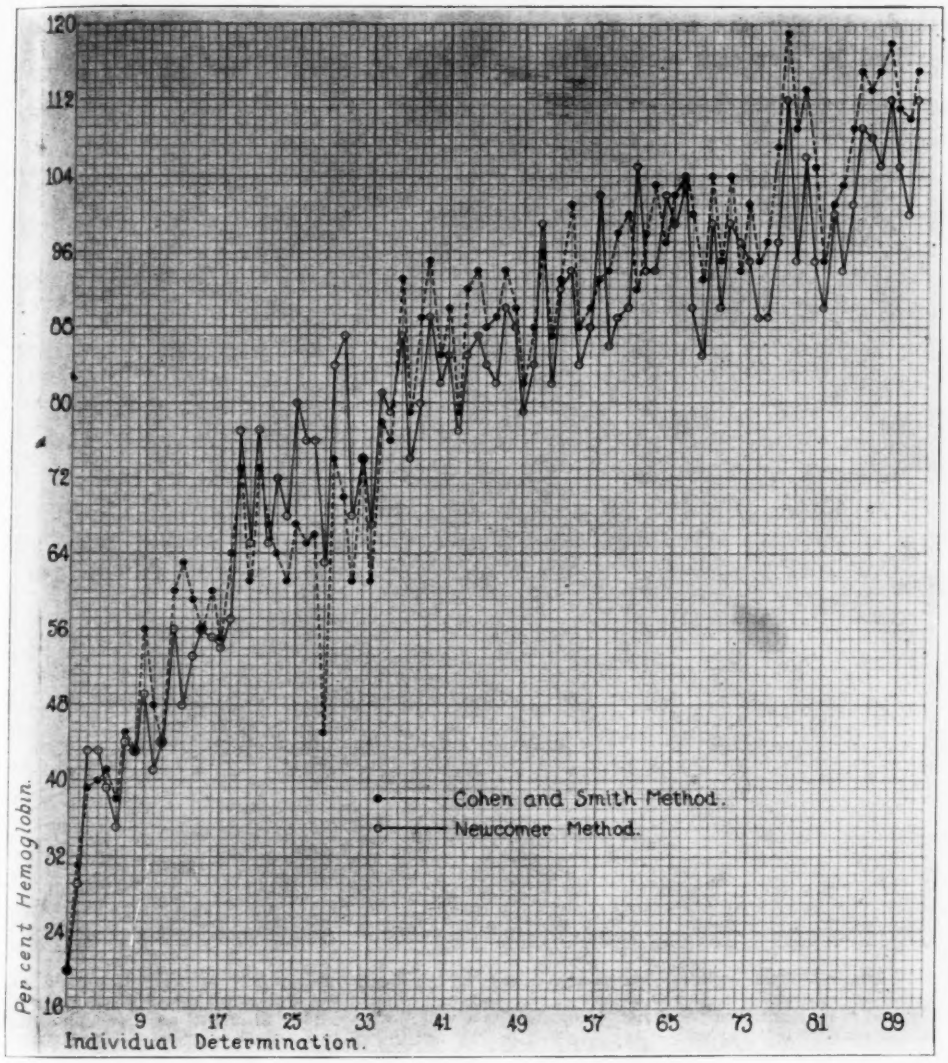

Fig. 4.—The marked uniformity between the Newcomer, and Cohen and Smith acid hematin methods is demonstrated. With but few exceptions, the variations are within the limits of error. From the clinical point of view these methods are equally accurate and interchangeable.

20 per cent aqueous solution of tannic acid, or a 1 per cent solution of gallic acid. After heating this for one minute, a dark brown mixture results. The dilution desired can then be made, and corresponds very closely to an acid hematin solution when viewed in a Duboscq colorimeter. Jacobson claims

that a 20 per cent solution has remained in the sunlight for ten months with no apparent change of color.

In 1916 Haessler and Newcomer devised another acid hematin hemoglobinometer adopting the principle of Sahli's instrument, but using eleven standard tubes arranged in a rack instead of but one. The readings on these tubes vary by 10, and represent hemoglobin readings from 10 to 110 per cent. The standard fluids are made up according to Sahli's specifications. The tube marked 100 per cent contains an equivalent in acid hematin of 17.2 gm. of

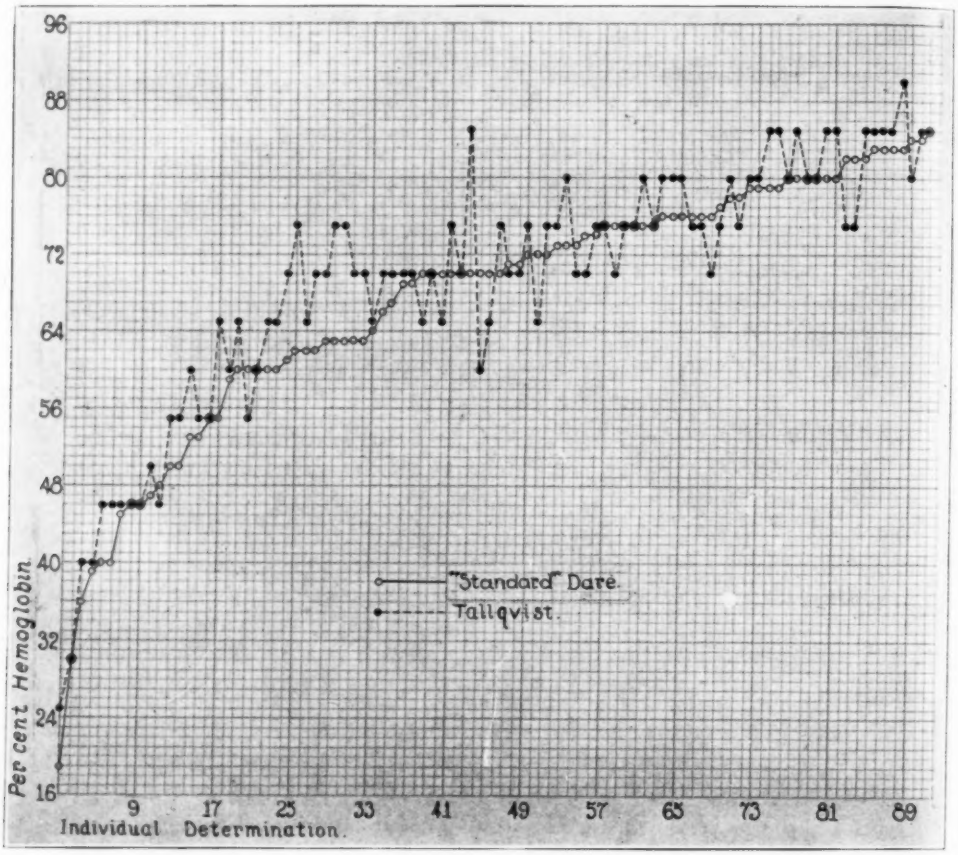

Fig. 5.—The striking similarity is shown between hemoglobin estimations made on the Tallqvist and Dare hemoglobinometers. This curve suggests strongly that if directions are followed with care, the Tallqvist determinations will be almost as accurate as those obtained on the standard Dare instrument.

hemoglobin in 10,000 c.c. The patient's blood is obtained in an ordinary, red blood pipette, a 1:100 dilution is made with 0.1 normal hydrochloric acid, and the solution compared with the standard tubes in the rack. The tube containing the blood to be tested, or the comparison tube, is inserted between the different tubes of the standard and moved one way or the other on the rack until the shading becomes harmonious, or as nearly so as possible. The per cent of hemoglobin is then read or estimated. As the readings on the

instrument vary by 10 per cent, the values are obviously only approximate. For this reason the percentages obtained are of value only as a comparison.

The method of determining hemoglobin described by Palmer, in 1918, is rapidly becoming popular, and is recognized as a standard method today. It is a comparison, by means of a colorimeter, of carbon monoxide hemoglobin solutions, one solution having a known hemoglobin content. The principle is similar to that described by Hoppe-Seyler in 1892. The standard solution is prepared as follows:

A quantity of ox or human blood is defibrinated. The oxygen capacity is determined according to the method of Van Slyke. The blood is then diluted with 0.4 per cent ammonium hydroxide solution so as to make a 20 per cent solution having an oxygen capacity of 18.5 volumes per cent. This solution is saturated with carbon monoxide by bubbling ordinary illuminating gas through it for ten minutes. It is immediately plugged, preferably with a glass stopper. This standard, 20 per cent solution should then be sealed and kept in the dark on ice. When used for hemoglobin estimation, a 1 per cent solution is made. Five cubic centimeters of this 20 per cent solution is diluted to 100 c.c. with 0.4 per cent ammonium hydroxide solution and saturated with carbon monoxide. A 1 per cent solution of a patient's blood is made by transferring 0.05 c.c. of blood into 5 c.c. of 0.4 per cent ammonium hydroxide solution. The blood pipette is rinsed by drawing the ammonia solution into it three or four times. Illuminating gas is then bubbled through the solution for thirty seconds. This solution is compared in a Duboscq colorimeter with the standard 1 per cent solution set at 10. The average of several readings is taken. The calculation is $\frac{10}{R} \times 100 =$ per cent of hemoglobin. The color match is excellent.

Considerable difficulty is met with in obtaining standards which will remain constant. Robscheit records changes in color value of Palmer standards of 10 to 20 per cent in a period of from seven to twelve months. If standards are prepared once a month, however, very accurate readings are obtained.[15] It is not always possible to obtain illuminating gas, which adds further difficulties.

In 1919, Newcomer presented his method of estimating hemoglobin by the use of a carefully prepared "high transmission yellow" semaphore glass disk. This instrument is based on the spectro-photometric properties of acid hematin. These thin glass disks of known thickness (about 1 mm.) and known hemoglobin values are used in place of the standard solution in the colorimeter. The disk is placed in the light path of one of the cups of the colorimeter (at the top of the plunger) and the corresponding cup is filled with water. In the other cup is poured the solution to be read. This consists of blood diluted with 0.1 hydrochloric acid. Five c.c. of 0.1 normal hydrochloric acid is measured with a pipette into the colorimeter cup or a small tube. Twenty c.mm. of blood is emptied and rinsed into this solution. The height of the color, produced by the acid hematin formed, appears in about forty minutes. The colors are then matched and the reading on the colorimeter is noted. A

scale accompanies the disk, and the hemoglobin percentage or the grams for each 100 c.c. is obtained by dividing the reading on the colorimeter into the appropriate figure in the scale. This scale is corrected both for time factor before matching, and for the thickness of the standard disk used. The estimations of hemoglobin are so satisfactory with this instrument that these disks are now made a part of the regular equipment of the Bausch and Lomb colorimeter of the Duboscq type. The new Bock and Benedict colorimeter is also well adapted for use with the Newcomer disk.

In 1919, Cohen and Smith described a practical method of accurately determining hemoglobin. Like Palmer, they use a colorimeter, but employ an acid hematin standard. The authors say that this method was devised for field use and has proved very satisfactory in the United States Army.

Preparation of Standard Solution.—About 50 c.c. of blood (ox or human) is defibrinated. The oxygen capacity is determined by the Van Slyke method. The blood is then diluted with 0.1 normal hydrochloric acid to make a 20 per cent stock solution with an oxygen capacity of 18.5 per cent. Such a standard will contain approximately 14 gm. of hemoglobin for each 100 c.c. This solution is treated with a few drops of chloroform to prevent mold formation, and is then placed on ice in a dark, glass-stoppered bottle. The comparison standard to be used in the colorimeter is a 0.5 per cent solution, prepared by diluting 2.5 c.c. of stock standard solution with 0.1 normal hydrochloride acid up to 100 c.c. By means of a calibrated Sahli pipette 0.02 c.c. of blood is obtained and added to 6 c.c. of 0.1 normal hydrochloride solution. The pipette is carefully washed several times by drawing the solution into it. The solution is then poured into one of the cups of the colorimeter and compared with the 0.5 per cent comparison standard. The average of several readings is accepted as correct.

With the standard solution a 0.5 per cent blood solution, and the Duboscq colorimeter set at 10, the calculation will be as follows:

$$\frac{1.5 \times 10 \times 100}{R} = \text{the percentage of hemoglobin.}$$

Cohen and Smith assert that the stock standard solution will remain stable at least three months. They find that accurate results are obtained by comparatively unskilled workers. The results are very accurate and the color match is good. Dilution errors must be carefully eliminated.

A method similar to that of Newcomer was described in the literature by Dreyer, Bazett and Pierce, in 1920. Dreyer says that for ten years he has used a hemolyzed solution of blood and compared it in a Duboscq colorimeter, using a colored glass as a standard. In making such determinations a 1:100 dilution of blood is made by diluting 0.1 c.c. of blood with 19.9 c.c. of normal saline. This solution is intimately mixed, and may be placed on ice until convenient to make the reading when it is hemolyzed by using a very minute amount of saponin. This can be done in a warm room by gently rotating the solution, but preferably by heating for thirty minutes in a water bath at about 30° C. The hemolyzed solution is then poured into one of the

cups of the colorimeter and matched against the pink glass standard. Artificial light is used, preferably one rich in yellow rays. The results are more constant if the readings are made in a dark room and when the reflector only is illuminated. The authors emphasize the variations in hemoglobin readings taken at different hours of the day, and regard the variations to be the smallest from 5:00 to 7:00 P.M.

The hemoglobin estimations made by the Cohen and Smith, Palmer, Newcomer and Dreyer methods, using a Dubosecq colorimeter, are similar and very accurate.

A COMPARISON OF VARIOUS METHODS OF HEMOGLOBIN DETERMINATION

The Dare instrument is used at the Mayo Clinic. Because the determinations were so low in apparently normal individuals, it was thought advisable to check the method as well as to ascertain the value of other methods

TABLE I

PALMER'S METHOD	NEWCOMER'S METHOD (GLASS 0.96 MM. THICK)	DIFFERENCE
per cent	*per cent*	
113	114	+1
116	114	−2
99	100	+1
107	108	+1
98	101	+3
117	118	+1
100	100	0
100	100	0
100	101	+1
100	103	+3
100	99.2	−0.8
Average difference		+0.73

for general use. No satisfactory investigations relative to the value of the Dare hemoglobinometer were found in the literature.

In this experimental work the Newcomer disk, the acid hematin method as described by Cohen and Smith, the Tallqvist hemoglobinometer, and two Dare instruments, a "standard" Dare hemoglobinometer and a "special"* Dare hemoglobinometer were used.

Patients of the Clinic sent to the hematology laboratory for hemoglobin determination and healthy Clinic workers were examined exclusively in this investigation.

Newcomer Disk Method.—The study by this method proved very satisfactory. The procedure is simple and the determination can be accomplished quickly. One disk, 1.0 mm. in thickness, was used. There was some difficulty at times in matching colors, especially when the hemoglobin readings were low. Light was supplied by a northern exposure and an electric light with Corning "daylight glass." The electric light with the daylight glass, being always constant, was thought superior to daylight. In Robscheit's

*Because of the findings in Fig. I, Dr. A. H. Sanford, of the Mayo Clinic, had a "special" Dare hemoglobinometer made by the Ricker Instrument Company, of Philadelphia, in which the glass prism comparison standard was ground 15 per cent thinner at the upper end of the scale than that of the "standard" Dare.

work it is noticed that the Palmer method was compared with the Newcomer disk, and that the readings were similar (Table I). The Palmer method is considered excellent, and these results tend to substantiate Newcomer's contention concerning the accuracy of his method. In addition Robscheit compared the Palmer method with the Van Slyke oxygen capacity method and found that the hemoglobin determinations were almost identical (Table II). The fact that the Palmer, Van Slyke, and Newcomer disk readings were so similar tends to prove that the Newcomer disk method is dependable. In

TABLE II

Hemoglobin

PALMER'S METHOD	AUTHOR'S MODIFICATION
per cent	*per cent*
75	74
70	70
77	76
77	77
74	73
73	73
74	74
75	74
76	75
73	73
64	64
63	63
76	76
45	45
60	60
84	85
69	69

TABLE III

VAN SLYKE'S OXYGEN CAPACITY METHOD	PALMER'S METHOD	AUTHOR'S MODIFICATION
per cent	*per cent*	*per cent*
113	113	114
117	118	117
99	98	99
124	124	124
109	108	109

general, with a disk of satisfactory thickness, the Newcomer method is very accurate and is of immense practical value, eliminating the preparation of standard solutions.

Cohen and Smith Method.—In determining the hemoglobin by this method, originally 0.20 c.c. of the blood to be examined was diluted with 6 c.c. of 0.1 normal hydrochloric acid. It was found early that the plunger of the colorimeter was often out of the acid hematin solution when the readings were low and only 6 c.c. of 0.1 normal hydrochloric acid was used. For this reason 0.40 c.c. of blood and 12 c.c. of 0.1 normal hydrochloric acid were always used thereafter, with no further difficulties.

Meyer and Butterfield, Newcomer and others claim that a maximal color depth is slow to appear in the acid hematin methods, and that the readings

vary from 2 to 20 per cent if the estimations are made too soon. Newcomer says that the readings can be made safely at the end of forty minutes. Therefore, in the methods involving acid hematin solutions, all readings were made in from six to twelve hours. Berman maintains that this difficulty can be overcome by boiling the acid hematin solution for one minute, allowing it to cool for one minute, and then making the estimation. Considerable criticism of acid hematin methods has arisen on account of the unstable properties of acid hematin standards. Using defibrinated blood and following the technic of Robscheit, or of Cohen and Smith, standards are obtained which remain uniform from four to seven months. If the 0.5 per cent standard is made up every two to three weeks and kept on ice, the readings should be very accurate. In this investigation fresh 0.5 per cent standard solutions were made up weekly, and the 20 per cent stock standard solutions every three months. The Duboscq colorimeter was used exclusively. Robscheit also compared her acid hematin method with the Palmer method and obtained similar readings (Table III).

Tallqvist Hemoglobinometer.—This instrument was used more out of curiosity than to determine the real value of the method. The directions were carefully followed with curious results. While the instrument is not accurate, the readings are at least approximate. According to Barker, "If it does nothing more than lead the practitioner to realize the necessity of seeing the color of drawn blood as it appears on filter paper, or on a towel, and to recognize the fallacy of trusting to the appearance of visible mucous membranes, it is in so far, praiseworthy."

RESULTS OF THE VARIOUS METHODS OF HEMOGLOBIN DETERMINATION USED

In the first study, represented by the curve in Fig. 2, 199 patients were examined. The hemoglobin values obtained on the Dare instrument were checked against those obtained by the acid hematin method as described by Cohen and Smith. As a result of these findings, further investigations were made, and ninety-one additional patients examined. In this work the Cohen and Smith method is considered the standard, and in the estimation of the accuracy of other hemoglobinometers used, the hemoglobin percentages obtained by these instruments are compared with those obtained by the Cohen and Smith method. The individual readings representing the percentage of hemoglobin calculated in each method are brought out in Figs. 2, 3, 4, and 5.

CONCLUSIONS

1. The standard Dare hemoglobinometer is a practical instrument for hemoglobin determinations from 20 to 60 or 65 per cent.

2. Above 70 per cent the hemoglobin estimations on the standard Dare instrument are very misleading.

3. The Newcomer disk method is a unique and practical method of estimating the percentage of hemoglobin in the blood. The percentages obtained are reliable.

4. The Cohen and Smith acid hematin method is accurate and fairly

practical and should be used in the estimation of the percentage of hemoglobin present in all suspected cases of anemia.

5. The special Dare instrument is a decided improvement over the standard Dare instrument.

6. The Tallqvist hemoglobinometer is probably quite as accurate as the standard Dare instrument.

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LABORATORY METHODS

THE DETERMINATION OF URIC ACID IN BLOOD BY THE METHOD OF FOLIN AND WU*

By Roger S. Hubbard, Ph.D., and Lucy L. Finner, B.S. (Chem.), New York

A NUMBER of investigators have recently discussed the determination of uric acid by the method described by Folin and Wu in 1919. Several of them (Jackson and Palmer, 1922; Morris and Macleod, 1922 a; Benedict, 1922; Folin, 1922) have suggested modifications in technic by which it is possible to obtain more color from a given amount of uric acid than could be obtained by the method as originally described. Pucher (1922 a) has stated that part of the uric acid present in blood is carried down with the precipitated protein, and (Pucher, 1922 b) has suggested a method for obviating this difficulty, as has also Folin (1922). Morris and Macleod (1922 a) have pointed out that reduced silver may be introduced with the reagents used for separating uric acid from interfering substances, and have suggested that it be separated as the zinc salt (discussed by Morris in 1916) instead of as the silver salt as recommended by Folin and Wu. Morris and Macleod (1922 b) have also described a reaction noted when potassium oxalate was added to blood; in a certain number of specimens they found higher values after this salt was added. They have attributed this increase to the formation of uric acid from some second compound present in the blood, which, from its chemical behavior, they have designated as a second form of uric acid. Benedict (1922) has discussed the theoretical and practical difficulties involved in carrying out the separation of uric acid from interfering compounds recommended by Folin and Wu, has described a method for the determination of uric acid without such a preliminary separation, and has given a table in which values obtained by this new method and by the method of Folin and Wu are compared with each other and with nonprotein nitrogen determinations done upon the samples of blood. Folin (1922) has also discussed this separation of uric acid from interfering compounds, and described a modification of the technic used for precipitating it.

In discussing the table of comparative results referred to above, Benedict points out that the method for determining uric acid directly in protein-free filtrates tends to give higher values than does the method of Folin and Wu upon blood with normal nonprotein nitrogen content, as would be expected

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if the method of separation employed in the earlier method failed to recover all the uric acid present. On the other hand he found that bloods containing increased amounts of nonprotein nitrogen sometimes gave higher results by the method of Folin and Wu than by the new method. He believes that there is some compound, not uric acid, in such bloods which carries through the method of purification and reacts with the reagent used in the method. Many results have been obtained by the method of Folin and Wu in various clinical pathologic laboratories, and it has seemed worth while to examine a series of such results to determine, if possible, to what extent these difficulties have affected the clinical interpretation.

In the series presented here variations in the degree of nephritis as determined by various tests have been used as a standard for estimating the significance of the uric acid determinations. Myers (1921) has recently summarized the work upon the retention of uric acid in this condition, and has concluded that increases in the amounts of uric acid in the blood regularly accompany increases in the amount of urea present. He has shown too that increased amounts of uric acid are found in the blood of nephritic patients when the condition has not advanced far enough to produce measurable increases in the amount of urea present. These findings have recently been confirmed by Czoniczer (1922).

The results discussed here include all of the determinations of blood uric acid on blood drawn before breakfast made in this laboratory during a period of two years and a half following the introduction of the method of Folin and Wu, except determinations carried out on three cases of gout (in which the expected high values were found) and those done on twenty-six cases in which no determinations of urea were made, and on which, therefore, there is not sufficient data available for determining approximately the degree of nephritis present. Total nonprotein nitrogen determinations were done upon the larger part of these twenty-six cases, and the results appeared to agree well with those discussed. No cases of leucemia were studied during this time.

Before proceeding with a discussion of the results certain facts concerning the cases studied and the difficulties recognized in the use of the Folin and Wu method should be mentioned. Most of the specimens were from patients who were considered as possible cases of nephritis, or who were suffering from heart disease or hypertension. In many instances they had been living upon diets low in protein for some time before they were admitted to the hospital, and a comparatively low concentration of urea in the blood was the rule. It has been suggested (Hubbard, 1922 a) that 18 or 20 mg. of urea nitrogen per 100 c.c. of blood should be regarded as the upper limit of normal in the fasting blood in such a series. Of the objections to the method discussed above, the difficulty in reading the color given by such amounts of uric acid as are found in normal blood and irregularities produced by the presence of varying amounts of potassium oxalate were the only ones recognized as markedly influencing the results, and of these the effect of potassium oxalate alone appeared to influence the clinical in-

terpretation of the values obtained. Precautions similar to those suggested by Folin and Wu were used in collecting the blood to insure the presence of a minimum amount of the salt, but it was not always present in the same concentration in all specimens. It was noticed that samples which form their behavior with the protein precipitants seemed to contain an excess of the anticoagulant appeared to contain relatively large amounts of uric acid. In determinations on other samples from the same cases smaller amounts were found when there was not the same reason to suspect the presence of an excess of oxalate. The addition of this salt before the removal of the proteins caused an apparent increase in the amount of uric acid present in some specimens. On the addition of silver lactate to such specimens a larger bulk of precipitate than usual was noticed. These results were in accord with the experimental work of Morris and Macleod, but a different interpretation was given to them than that which those authors have given. It was thought that all of the uric acid was not recovered in some cases when only minimum amounts of potassium oxalate were present, and accordingly the probable borderline between normal and increased amounts of uric acids was placed at the low figure of 2.5 mg. per 100 c.c. (Hubbard, 1923) and a conservative interpretation was placed upon results somewhat in excess of this figure.

TABLE I

UREA NITROGEN RANGE MG. PER 100 C.C.	NO.	URIC ACID RANGE—MG. PER 100 C.C. TO 1 NO.	1-1.5 NO.	1.5-2 NO.	2-2.5 NO.	2.5-3 NO.	3-3.5 NO.	3.5-4 NO.	4-5 NO.	5-6 NO.	6-8 NO.	8-10 NO.
to 12	80	3	10	19	12	6	12	7	6	2	3	0
12-14	65	1	3	9	15	12	9	6	6	2	1	1
14-16	25	0	0	7	4	6	3	1	2	1	1	0
16-18	25	0	3	3	7	2	2	4	2	1	1	0
18-20	7	0	1	3	0	0	0	0	2	1	0	0
20-22	7	0	0	0	3	2	1	1	0	0	0	0
22-25	3	0	0	0	0	1	0	0	1	1	0	0
25-30	5	0	0	0	1	1	1	1	0	1	0	0
30-40	4	0	0	0	0	0	0	1	1	1	1	0
40-50	3	0	0	0	0	0	0	0	1	2	0	0
50-70	8	0	0	0	0	0	0	1	1	2	4	0
70-100	4	0	0	0	0	0	0	0	0	1	3	1
100+	3	0	0	0	0	0	0	0	0	1	1	1

In Table I the different levels of blood urea nitrogen (determined approximately in the way described by Van Slyke and Cullen in 1914) have been grouped together, and the number of cases in each group which showed different amounts of uric acid in the blood given. Inspection of the table shows that in general the percentage of cases which gave high values for the uric acid content of the blood increased as the values of the urea nitrogen increased, but that there were many exceptions to such a statement. Four samples of blood which contained more than 20 mg. of urea nitrogen per 100 c.c. of blood contained 2.5 mg. or less of uric acid, and four more contained between 2.5 and 3 mg. These results cannot be easily explained except on the basis of the failure of these cases, which probably were suffer-

ing from nephritis (the excretion of phenolsulphonephthalein was decreased in four of the eight cases, and was normal in two) to show a retention of uric acid, or of failure to recover all of the uric acid present by the technic used. More cases seemed to show larger amounts of uric acid than the urea content of the blood would indicate, but these cases include mild cases of nephritis in which the urea content is not increased. To determine the percentage of these cases which can properly be classified as cases of early nephritis the figures found by the phenolsulphonephthalein test (Rowntree and Geraghty, 1919) are given in Table II. Frontz and Geraghty (1922)

TABLE II

URIC ACID RANGE MG. PER 100 C.C.		PHENOLSULPHONEPHTHALEIN TEST RANGE—PER CENT			
		70+	50-70	30-50	TO 30
	NO.	NO.	NO.	NO.	NO.
2.5-3	26	24	2	10	10	2
3-3.5	26	16	2	7	6	1
3.5-4	18	10	0	4	5	1
4-5	18	15	1	6	7	1
5-6	7	7	2	1	4	0
6+	6	4	0	0	3	1

Urea nitrogen concentration less than 20 mg. per 100 c.c. of blood.

and Hubbard (1922 a) have shown that a decreased excretion of this dye can be detected in cases of nephritis before an increased retention of urea can be demonstrated. Table II shows that seventy-five per cent of the cases with high uric acid, but low urea concentrations in the blood were tested by the phenolsulphonephthalein test, and more than fifty per cent showed a diminished excretion of the dye, but that some cases with values as high as 5 mg. per 100 c.c. excreted normal amounts. It has been shown (Hubbard, 1922 b) that some cases which show symptoms of nephritis excrete normal amounts of the dye, but show evidences of kidney impairment when tested by the specific gravity fixation test of Mosenthal (1915). Such tests were done upon some of the patients with increased amounts of uric acid in the blood, but normal rates of dye elimination and blood urea concentrations. The results are given in Table III.

TABLE III

URIC ACID RANGE MG. PER 100 C.C.		SPECIFIC GRAVITY FIXATION TEST			
		PER CENT AT NIGHT 45 AND MORE	SP. GR. DIFFERENCE 0.010 AND LESS	NEGATIVE	
	NO.	NO.	NO.	NO.	NO.
2.5-3	12	7	1	3	3
3-3.5	9	7	4	4	2
3.5-4	4	4	1	3	1
4-5	7	4	3	3	0
5-6	3	2	1	0	1
6+	0	0	0	0	0

Urea nitrogen concentration less than 20 mg. per 100 c.c. of blood.
Phenolsulphonephthalein excretion greater than 50 per cent in two hours.

Inspection of the Table shows that seventy per cent of the cases tested excreted forty-five per cent or more of the urine at night, or showed a dif-

ference between the specific gravities of different specimens of 0.010 or less. Both of these findings are commonly found only in nephritic patients, and therefore it can be said that in only a relatively small percentage of cases which contained increased amounts of uric acid in the blood further evidence of nephritis could not be found by other quantitative or semiquantitative tests, but there were many cases upon whom complete studies were not made.

A study of the uric acid values obtained in this series has shown that increases in the urea concentration of the blood were usually accompanied by increases in the uric acid concentration. In the larger part of the cases which showed increased retention of uric acid but normal concentrations of urea some degree of renal impairment could be demonstrated by other tests, but there were some cases in which such impairment could not be demonstrated; there was no reason for believing that these patients were suffering either from gout or from leucemia. A small number of cases failed to show any retention of uric acid when urea retention was found. The figure regarded as the upper limit of normal was placed at the low value of 2.5 mg. of uric acid per 100 c.c. of blood, and the study has shown that when values between 2.5 and 3.0 mg. were found, slight evidences of nephritis may or may not be given by other tests. In general it may be said that the presence of some degree of nephritis could be demonstrated in about half the cases in which these borderline figures were found.

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A STUDY OF THE RELATION BETWEEN THE PRESENCE OF INDICAN AND OF URORoseINOGEN IN THE URINE IN CERTAIN DISEASES*

By Jacob Rosenbloom, M.D., Ph.D., Pittsburgh, Pa.

I. INTRODUCTION

UROROSEINOGEN[1] was shown by Herter to be indol-acetic acid. Indican is the potassium salt of indoxyl-sulphuric acid. One would think that in conditions of an increased excretion of indican that indol-acetic acid would also be excreted in larger amounts than normal. This study was undertaken to discover if this were so.

II. METHODS

The indican was tested for by the method described by Rosenbloom,[2] using 10 c.c. of urine from a 24-hour specimen, 10 c.c. Obermyers reagent and 5 c.c. of chloroform, the extent of the bluing of the chloroform determining the gradation of the indican present.

The ururoseinogen was tested for by mixing 10 c.c. of urine with 10 c.c. of C.P. hydrochloric acid, adding five drops of a freshly prepared one per cent solution of sodium nitrite and noting the deepness of the rose-red color that appears when ururoseinogen is present.

Following are the cases studied:

Table I

CASE NO.	DIAGNOSIS	INDICAN	UROROSEINOGEN
1.	Diabetes	++++	++
2.	Acute glom. nephritis	++	+++
3.	Gastric ulcer	+	++
4.	Cancer of breast	++	Faint
5.	Typhoid	+++	++
6.	Diabetes	0	+
7.	Pneumonia	++++	++++
8.	Pneumonia	0	+
9.	Chr. cholecystitis	0	+
10.	Bichloride poisoning	+	Faint
11.	Pneumonia	++	++++
12.	Typhoid	0	++
13.	Indicanuria	++++	Faint
14.	Chr. nephritis	+++	Faint
15.	Chr. endocarditis	++	++
16.	Arteriosclerosis	0	+++

III. CONCLUSIONS

From a study of this table it may be noted that there is no constant relation between the excretion of indican and ururoseinogen. At times with an increased indican there is present an increased ururoseinogen, but however with a persistent indicanuria of marked degree there is often only a faint trace of ururoseinogen present. However occasionally they do parallel each other.

*Received for publication, February 26, 1923.

As they both have the same significance as an indicator of intestinal putrefaction, I would draw attention to the necessity of testing for both substances in the urine when using these tests as an indication of intestinal putrefaction.

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AN EMERGENCY MICROTOME*

By Arthur H. Glaister, Technician, Florence, Alabama.

IN the absence of a microtome, passable sections can be made in the following manner:

Place the microscope beside a steady shelf of suitable height, remove the eye piece and fit the draw tube with a cork having a coin cemented on top with sealing wax.

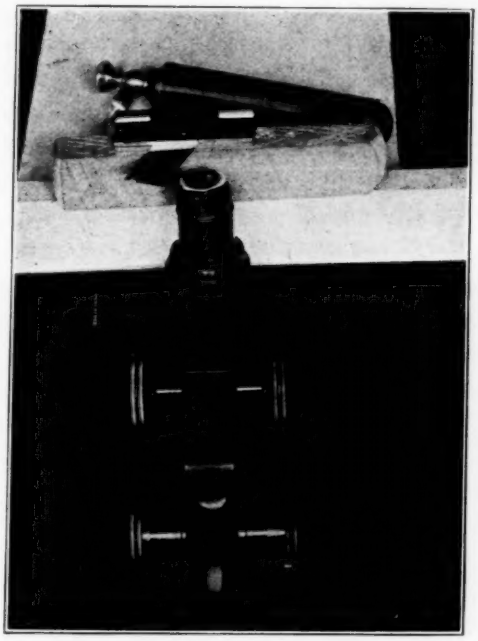

Fig. 1.

For the knife use a safety-razor blade clamped in a folded hinge which is secured to a block of fairly hard wood with wood screws.

Freeze the tissue on the coin with a spray of ethyl chloride and cut by sliding the knife block along a piece of glass secured to the edge of the shelf, adjusting the tissue to the knife with the coarse focus and feeding the desired thickness between cuts with the fine focus.

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GASTRIC ANALYSIS AND THE CONSTANCY OF THE PERCENTILE RELATIONSHIP AMONG THE TITRABLE FACTORS OF THE GASTRIC SECRETION*

(Preliminary Paper)

By B. S. Levine, Ph.D., Waukesha, Wis.

GASTRIC analysis has failed frequently to be of the desired assistance to the diagnostician, and for this reason it has fallen into ill repute with many a physician, especially since the development of the x-ray technic and the spectacular results it affords. A thorough search of the situation, however, reveals that the fault rests not with the inadequacies of the results of the gastric analyses, but with our inability to interpret the results and to properly associate them with the general clinical conditions. Rehfuss and Hawk have stated justly that it is erroneous to conclude that a certain degree of acidity is indicative of a definite pathologic condition just because a large percentage of cases with such a condition possessed a similar degree of acidity. Many instances have come under the writer's observation in which cases with a total acidity of about 65°, a total free acidity of 45°, and a total physiologically active acidity of 51° were designated by the attending physician as "Hypoacidity" and HCl preparations were prescribed, whereas others have designated as "normal acidity" cases in which the total acidity was between 22° and 12°, the total free acidity was between 13° and 7°, and the total physiologically active acidity was between 20° and 10°.

Lack of reliable information concerning the proper limits of high and low acidity in normal individuals was another source which added to the confusion prevailing in the gastric analyses. This, however, has been adequately established by Rehfuss and Hawk and their collaborators. It is well known now that there are normal individuals with low acidity as well as with high, and the mere determination of the degrees of the various acidities for this reason adds little of value in the attempt to establish the gastric status of individual cases.

With the view to finding some factors of value in such cases, the writer has carried out gastric analyses on a number of individuals, and prolonged series on the same individuals. The meal used in all cases was the so-called Ewald meal, consisting of a piece of butter-free toast and a glassful of water, and which was removed by siphoning or by the use of aspiration, as the case required, one hour after the ingestion of the meal. The titrable factors were

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determined by titrating 10 c.c. of the unfiltered gastric contents with N/10 NaOH against the following indicators: phenolphthalein, alizarine sodium monosulphonate, and dimethylaminoazobenzol. The titration was performed immediately after the removal of the gastric secretion from the stomach. Like the above-cited investigators, the writer has found that high degrees of acidity are met with in cases whose history and microscopic examination of the gastric content are negative. Low degrees of acidity, at first glance of a seemingly pathologic character, were also obtained from individuals with no pathologic complaints. Individual cases were then followed up from day to day in an attempt to determine the constancy of volume and of the degrees of the various acidities in the same individuals, assuming that in normal cases these findings would be more or less positive, whereas in the cases involving gastric pathology the regularity would be upset. The assumption proved a mistaken one. Volumes and degrees of acidity varied frequently in the same normal individuals sufficiently to render this point valueless for diagnostic purposes.

The relationship which might exist between the titrable factors was then thoroughly studied. After numerous mathematical calculations, the following simple method of reporting gastric titrable factors was adopted. It revealed interesting and instructive information. Total acidity was reported in absolute degrees of acidity as usual, and was also designated as 100.0 per cent. The other factors were reported in terms of absolute degrees of acidity as determined by titration and in terms of their percentage of the total acidity. Over fifty cases have been studied in this way thus far, but only several examples of actual analyses are given below:

PATIENT A. K. D.

	9-19-'22	9-22-'22	9-23-'22
Total acidity	22°—100.0%	12°—100.0%	35°—100.0%
To. free acidity	13 — 59.0	7 — 58.3	21 — 60.0
Combined HCl acidity	9 — 41.0	5 — 41.7	14 — 40.0
Free HCl acidity	13 — 59.0	7 — 58.3	21 — 60.0
To. physiol. active acidity	22 —100.0	12 —100.0	35 —100.0
Organic acidity	00 —000.0	00 —000.0	00 —000.0

The noteworthy points in connection with the results of the above analyses are the low degrees of the acidities, the great variations manifest in the absolute figures, and the constancy of the percentile relationships. Microscopic findings in this case were negative.

The constancy of the percentile relationship of the titrable factors in the gastric secretion as manifest after the Ewald meal is not a temporary one, but is apparently typical of the individual, as may be seen from the following analyses of gastric content taken at considerably remote dates:

PATIENT J. K. C.

	12-31-'21	1-17-'22	9-13-'22
Total acidity	52°—100.0%	65°—100.0%	60°—100.0%
Total free acidity	32 — 61.5	45 — 69.2	40 — 66.7
Combined HCl acidity	20 — 38.5	20 — 30.8	20 — 33.3
Free HCl acidity	21 — 40.4	31 — 47.3	28 — 46.7
To. physiol. active acidity	41 — 78.9	51 — 78.1	48 — 80.0
Organic acidity	11 — 21.1	14 — 21.9	12 — 20.0

There seems to be some difference in the Ewald meal administered on dates distantly apart. In such cases the percentile values of the titrable factors occasionally differ somewhat, but the percentile value of the total physiologically active acidity remains constant. The following analyses demonstrate this point:

PATIENT C. W.

	11-15-'21	9-20-'22
Total acidity	44°—100.0%	54°—100.0%
Total free acidity	40 — 90.0	33 — 61.1
Combined HCl acidity	4 — 9.1	21 — 38.9
Free HCl acidity	30 — 70.0	21 — 38.9
To. Physiol. active acidity	34 — 79.1	42 — 77.8
Organic acidity	10 — 20.9	12 — 22.2

However this may likewise be due to differences in the protein concentration of the gastric secretion itself, and may also prove of diagnostic value.

As far as the work has progressed now, it has indicated that in certain pathologic gastric conditions the constancy in the percentile relationship of the titrable factors is disturbed, and thereby gives warning to the attending physician. Certain deviations from this constancy are observed also in purely nervous conditions, especially in the so-called "anxiety neuroses," but the deviations are not as pronounced and seem to affect the percentile value of total physiologically active acidity only to a slight extent.

On the basis of the work done by us thus far, we have come to the following tentative conclusions regarding the titrable factors of the gastric analysis:

1. That there exists a constant percentile relationship between the titrable factors of the gastric secretion in the normal stomach.

2. That the Ewald meal test is the most convenient and in practically all cases with which the ordinary practitioner has to contend is a sufficient means for the determination of the gastric status of the patient from the physiologic and so-called functional points of view.

3. That high or low acid values may be typical of the individuals under investigation, and, hence, are to be considered normal in such cases, this point being a corroboration of the conclusion of Rehfuss, Hawk, and their collaborators.

4. That variations in the degrees of the acidities even to a marked extent may occur on consecutive days in the gastric contents of the same individuals with no pathologic gastric involvements.

5. That a series of at least three gastric analyses of the same type shall be performed on successive days, and the constancy of the percentile relationship of the titrable factors should be determined before any conclusion or opinion is formed regarding the case studied.

This preliminary paper is given publicity with the hope that it will stimulate interest and further investigation along the line of *the constancy of the percentile relationship of the titrable factors of the gastric secretion.* The detailed report of the work done by the writer in this line will be published in the future.

THE ESTIMATION OF ALBUMIN IN URINE*

By Armand J. Quick, Ph.D., Philadelphia, Pa.

THE simplest and best known clinical method for the estimation of albumin in urine is that of Esbach[1] which depends on the precipitation of albumin by a picric acid-citric acid reagent in a tube so calibrated that the height of precipitate after standing 24 hours can be directly read in terms of grams of protein per liter. In spite of all criticisms,[2] condemnations, and proposed modifications, the original method is still widely used, although it is well recognized that the results often are not even roughly quantitative. The greatest source of error lies in the tube itself. Since the method calls for an empirically calibrated tube, the specifications of which were never published, the original tube has been copied and recopied with ever accompanying errors so that now a variety of tubes are found on the market which vary greatly in shape and dimensions. The method, moreover, is empirical and takes little account of such factors as temperature and the specific gravity of the urine.

While numerous modifications have been proposed[2] it seems that none offer any distinct improvement either as to increasing its accuracy or its simplicity. With the aim of securing greater accuracy without destroying the simplicity of the procedure the following investigation was undertaken.

Since none of the Esbach tubes available were found satisfactory a 20 cm. tube rounded on the bottom without tapering, and having an internal diameter of 12 mm. was selected.† It was graduated in 0.5 c.c. up to 10 c.c. and further calibrated for 15 c.c.

In order to calibrate the new tube like the original Esbach in grams of albumin per liter, 10 c.c. of urine containing a known amount of egg albumin was transferred to the tube and treated with 5 c.c. of Esbach reagent (1 part picric acid, 2 parts citric acid, dissolved in 100 parts of water). After thorough mixing, the tube was allowed to stand for 24 hours at 21° C. Table I records the reading of the height of the precipitate in c.c. obtained with varying amounts of albumen.

In a similar way two other protein precipitating agents were studied. The first was Tsuchiya's reagent (1 part phosphotungstic acid and 5 parts conc. hydrochloric acid in 100 parts of 95 per cent alcohol) which was recently highly recommended by Pfeiffer,[3] the second was a 10 per cent solution of trichloracetic acid which, heretofore, has not been used as a substitute for the Esbach reagent. A comparison of the data obtained by the analysis

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†The tubes were kindly furnished by the Kimble Glass Co.

of urines containing known amounts of egg albumin with the different reagents may be made by referring either to Table I or Chart 1.

TABLE I

CONC. OF ALBUMIN G. PER LITER	READING IN C.C. ESBACH	READING IN C.C. TSUCHIYA	TRICHLORACETIC ACID
0.5	0.6	0.8	0.5
1.0	1.2	1.6	1.1
1.5	1.7	2.4	1.6
2.0	2.2	3.0	2.1
2.5	2.6	3.4	2.6
3.0	3.0	3.8	3.2
3.5	3.3	4.2	3.7
4.0	3.6	4.5	4.2
4.5	3.9	4.8	4.6
5.0	4.2	5.0	5.0

Specific Gravity of urine 1.013-1.015. Temperature 21-22° C.

The effect of temperature and of the specific gravity of urine was studied next, since these are the most important variable factors in the analysis. In studying the influence of temperature the albumin was precipitated in the usual way, using the three different reagents. One set of tubes was allowed to stand for 24 hours at room temperature (21-22° C.), another at 25° C., and the third at 10-11° C. The results are recorded in Table II.

TABLE II

VARIATIONS OF HEIGHT OF PRECIPITATE WITH TEMPERATURE

REAGENT	G. OF ALBUMIN PER LITER 10-11° C.	21-22° C.	26°
Esbach	3.2	2.4	2.0
Tsuchiya	5.	2.9	2.8
Trichloracetic	3.3	2.9	2.8

Urine contained 2.9 parts albumin per liter. Sp. G. 1.020.

To determine the effect of specific gravity on the height of the precipitate, a series of urines of different specific gravities were used, and a known amount of egg albumen was added to each specimen. In order to get the high specific gravity values, urea was added, while the lower values were obtained by dilution. The results are recorded in Table III.

TABLE III

EFFECT OF SPECIFIC GRAVITY ON THE HEIGHT OF THE PRECIPITATE

	G. OF ALBUMIN PER LITER SPECIFIC GRAVITY					
Reagent	1.003	1.012	1.017	1.022	1.028	1.045
Esbach	3.2	2.5	2.3	2.4	2.0	2.4
Tsuchiya	3.9	3.5	3.0	2.9	2.8	2.9
Trichloracetic acid	2.8	2.9	2.8	2.8	2.8	2.7

Temperature 20° C., conc. of albumin 2.9 g. per liter.

The results from all the experiments clearly point to the superiority of trichloracetic acid as a precipitating agent. In the first place the height

of the precipitate is very nearly directly proportional to the concentration of albumin within the limits of .05-.5 per cent; consequently there is less error in the higher readings than with either of the other two reagents. Fortunately, the Esbach and trichloracetic acid curves are nearly identical up to 0.3 per cent albumin so that the two can be used interchangeably for urines with low concentration of albumin. The greatest advantage in the use of trichloracetic acid lies in the fact that the effects of temperature and of the specific gravity of urine are reduced to a minimum. The precipitates obtained both with the Esbach's and Tsuchiya's reagent are effected by temperature, and the Esbach especially gives readings which are too low if the temperature is much above 20° C. Variation of specific gravity decidedly influences the volume of precipitate with Tsuchiya's reagent, the reading taken at a specific gravity 1.010 – 1.013 being about 30 per cent higher than at 1.020 – 1.025.

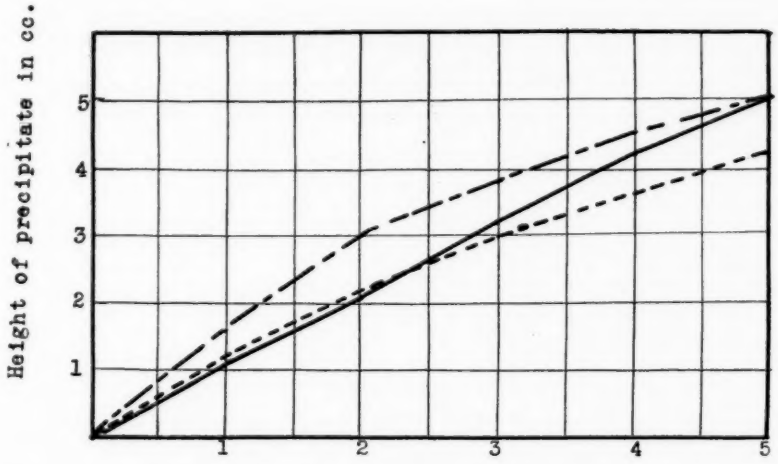

Chart 1.—Grams of albumin per liter. - - - - - Esbach - . - . Tsuchiya ——— Trichloracetic acid.

The method was next applied to pathological urines. The procedure was carried out as follows: 10 c.c. of urine were transferred to the tube, previously described, 5 c.c. of the reagent added, the tube stoppered and inverted 10 times to insure complete mixing. After allowing the tube to stand at room temperature for 24 hours the height of the precipitate was read and converted to grams of albumin per liter by means of Table I. In the case of urines containing more than 5 gm. of albumin per liter, only 5 c.c. of urine were taken and diluted with 5 c.c. of water.

Alkaline urines were acidified with conc. hydrochloric acid until acid to litmus. Both Tsuchiya's reagent and trichloracetic acid are sufficiently acid to effect complete precipitation in all but exceedingly alkaline urines, but with Esbach's reagent it is necessary to neutralize carefully excess alkalinity. The values obtained were compared with the more accurate coagulation method which was carried out in the following manner: 50 c.c. of urine was slightly acidified with acetic acid, and heated on a water-bath

until the coagulation of protein was complete. The precipitate was removed by centrifuging and after thorough washing was analyzed for nitrogen by the Kjeldahl method. By means of the factor 6.25, the grams of nitrogen found were calculated in terms of grams of protein. By using trichloracetic acid and following the method as outlined, it is possible to obtain results which are more accurate than those obtained by the use of either Esbach's or Tsuchiya's reagent. It might be remarked in passing that fresh urine should be used. The albumin readily undergoes decomposition if the urine is not well preserved, and even in well preserved specimens of urine, the albumin gradually undergoes changes so that none of the determinations mentioned will give satisfactory results.

TABLE IV

ANALYSIS OF URINES

Reading, g. of albumin per liter

ESBACH	TSUCHIYA	TRICHLORACETIC ACID	GRAVIMETRIC	SPECIFIC GRAVITY
2.6	3.2	2.7	2.7	1.016
0.8	0.7	0.6	0.8	1.012
4.5	3.2	3.2	3.2	1.010
1.0	1.0	0.9	0.8	1.012
2.6	2.6	3.0	3.5	1.010
2.6	3.0	3.2	3.2	1.016
1.2	1.5	1.1	1.1	1.010
9.6	10.0	7.0	6.6	1.006
4.3	3.2	2.8	3.1	1.010
1.8	3.5	2.0	1.8	1.013
1.0	1.4	1.0	1.1	1.005
7.6	10.0	6.8	6.7	1.020

SUMMARY

1. A comparative study of the modifications of the Esbach method for the determination of protein in urine has been made. Since the available Esbach tubes were unsatisfactory, a new tube was designed and described. Esbach's reagent, Tsuchiya's reagent, and a 10 per cent trichloracetic acid were studied as precipitating agents. The influence of temperature and of specific gravity of urine were determined.

2. A 10 per cent solution of trichloracetic acid as a precipitating reagent was shown to yield the most satisfactory results.

The author wishes to express his thanks to Dr. A. D. Waltz for furnishing the pathologic specimens of urine.

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A CLINICAL TEST FOR LIVER FUNCTION*

By Emil Bogen, Cincinnati, Ohio

EVIDENCE is rapidly accumulating that the removal of phenoltetrachlorphthalein from the blood stream, as first shown by Abel and Rowntree in 1910, is an index of the functional efficiency of the liver. Much credit is due to Sanford M. Rosenthal for developing a reliable method for the determination of this excretion, and for securing practical standards for the interpretation of the results. Unfortunately, the repeated withdrawals of relatively large amounts of blood, the danger of complete nullification of the readings by the slightest traces of hemolysis, the necessity for preparing fresh standards for each test made, and the consequent difficulty in preparing enough standards for accurate readings, are factors which retard the general adoption of this very ingenious and valuable procedure.

In order to make this test comparable in simplicity and ease of execution with the well-known phenolsulphonephthalein test for kidney function, the

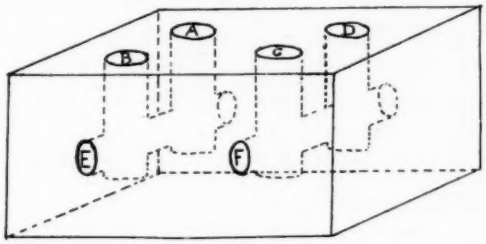

Fig. 1.

method of bicolorimetric comparison with permanent standards of a single sample of blood taken one hour after the injection of the dye was attempted, and the following modified technic was found, in a series of cases at the Cincinnati General Hospital ,to give eminently satisfactory results.

The patient is weighed and the dosage determined on the basis of five milligrams of phenoltetrachlorphthalein (or 0.1 c.c. of the sodium phenoltetrachlorphthalein solution put up by Hynson Westcott & Dunning of Baltimore, Md.,) for each kilogram of body weight. This dose is drawn up into a 25 c.c. Luer syringe, and sterile physiologic salt solution added to the 25 c.c. mark, and is then injected slowly through a narrow bore needle into a large vein on the right arm, using a tourniquet if needed to bring out the vein, but releasing it before the injection is commenced, and taking great care that the needle is freely in the vein, so that no extravasation may occur.

Exactly one hour later five to ten c.c. of blood is taken from the left arm, with a dry needle into a clean dry test tube, the blood is allowed to clot and the serum to separate out. We have been able to secure clearer sera,

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with less danger of hemolysis, by allowing the blood to clot with the test tube in a slanting position, and aspirating the clear serum that separates after several hours by means of a long narrow syringe such as is used in giving tuberculin. The serum obtained is divided into two small test tubes, 1.5 cm. in diameter, and two drops of ten per cent sodium hydroxide are added to one tube to bring out the color of the dye, which remains absolutely colorless without this alkalinization in the other tube, thus serving as a control, and the percentage of the dye present is determined by comparing with a set of standard solutions of the dye, through a two-color colorimeter.

A simple bicolorimeter is made as follows: A block of wood 2″ by 2″ by 2″ is taken and four holes are bored perpendicular to one surface just large enough to admit easily the small test tubes used to a depth of three or four centimeters, as shown in Fig. 1, A, B, C, and D.

Two holes are then bored horizontally exactly through these two pair of holes, with slightly smaller diameter, (Fig. 1, E and F) so that, by looking through one of these latter holes one might see through the two tubes standing in the corresponding vertical holes. A piece of ground glass or translucent paper is fastened over the back of the box, to facilitate the readings.

An empty tube is placed in hole A, the alkalinized serum in hole B, the control serum without alkali in hole C, and the standard solutions are then successively placed in hole D until one is found which, on looking through hole F, will yield the same color obtained by looking through hole E.

The standard solutions are made by first making a solution containing ten milligrams of dye in 100 c.c. of distilled water, and then making dilutions of 1, 2, 3, 4, 5, 6, 8, 10, 12½, 15, 17½, and 20 per cent of this first standard with distilled water containing a few drops of 10 per cent sodium hydroxide to bring out the color, and placing these diluted standard solutions in twelve small test tubes, 1.5 cm. in diameter, which are then sealed and marked and kept as permanent standards.

The following table is included to indicate the clinical significance of the one hour readings in a series of cases so tested at the Cincinnati General Hospital.

Erythema multiforme	1%
Pleurisy	1½%
Arsenic poisoning	4%
Carcinoma, abdominal	5%
Carcinoma of liver (?)	7%
Banti's Disease (?)	8%
Cirrhosis of liver	8%
Toxemia of pregnancy	10%
Cirrhosis of liver	10%
Catarrhal Jaundice (?)	14%
Cirrhosis of liver	20%
Cirrhosis of liver	22%

Dr. Rosenthal reports 37 cases, with readings at one hour as follows:

Carcinoma of liver	9-18%
Cirrhosis of liver	7-20%
Acute Hepatitis	20-22%
Liver abscess	9-10%
Toxemia of pregnancy	12%
Normals	0- 1½%
Nonhepatic diseases	1- 3%

We may infer from these figures that a reading of less than three per cent definitely testifies to a normally functioning liver, that from 4 to 8 per cent indicates partial hepatic insufficiency, and that a retention of over 8 per cent of the dye an hour after injection represents a pronounced liver impairment.

A USEFUL URINARY FINDING IN THE DIAGNOSIS OF HYDRONEPHROSIS AND PYONEPHROSIS*

By Jacob Rosenbloom, M.D., Ph.D., Pittsburgh, Pa.

IN three cases of pyonephrosis and in two cases of hydronephrosis, I have found globules of fat in the urine. I think the reason this finding has not been described before is due to the fact that in examining urine microscopically, the sediment is obtained by centrifuging or by allowing the urine to stand until a deposit forms. Fat globules being lighter than urine, come to the surface under these conditions and one must examine the surface layer of the urine to detect the same.

These globules are usually large and strongly refractile and stain black with osmic acid and red with Sudan III. They are also soluble in ether.

It will be recalled that fat in the urine, the so-called "lipuria" is always pathologic, as no free fat is found in normal urine. One must be sure that the fat is not due to contamination from a lubricant used in the catheterization or from the container. There also occurs the so-called alimentary lipuria due to too large an amount of fat in the diet. Webster states that pathologically it has been observed in various cachectic conditions, in crushing injuries, especially of the bones, in eclampsia, in chronic heart disease, fatty tumors, diabetes mellitus, tuberculosis, various affections of the pancreas and liver, nephritis, and after the use of various general protoplasmic poisons. In these cases the blood may also contain an excess of fat. It would be interesting to know if there is present an increased amount of fat in the blood in hydronephrosis and pyonephrosis.

There also exists the parasitic cyturia due to filaria and the nonparasitic cyturia of unknown origin and a lipuria due to inunctions of oil.

In one of the cases mentioned in this paper the fat droplets were only found in the urine obtained on catheterization of the diseased kidney. The catheterized urine from the normal kidney was free of the fat droplets.

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EDITORIALS

The Possibility of Extending Human Life

UNDER the above heading Dr. Louis I. Dublin, statistician for the Metropolitan Life Insurance Company, presented to the Harvey Society of New York in December, 1922, a most valuable paper. In the first place, Dublin points out that the mortality statistics which are now at our disposal are in no way satisfactory. They are, very properly called, crude death rates, and are likely to lead us into false conclusions. Age has a great influence on death rates and when we compare the mortality of New York City with that of Seattle this must be taken into consideration. If there are more babies and more old folks proportionately in New York City than there are in Seattle, quite naturally the death rate in the latter will be smaller than in the former, since the highest mortality is in the two extremes of life.

The article discusses present time longevity. The first reliable statistics along these lines are those for England and Wales covering the period between 1838 and 1854. At that time the average length of life in these countries was nearly forty-one years. During the following decades there

was a constant gain. The seventh table covering the period from 1910 to 1912 gave an average extension to fifty-three and one-half years. This shows a gain in the interval of seven decades of twelve and one-half years. On the European continent the best historical data are for Sweden. The first table covers the period from 1816 to 1840 and shows an expectation of life of forty-one and one-half years. From 1901 to 1910 the expectation of life in Sweden had increased to fifty-five and three-fourths years, thus showing a gain of fourteen and one-fourth years in an interval of eight decades. In our own country the first reliable table came from Massachusetts in 1855. At that time the expectation of life in Massachusetts was about forty years —the same as in England and Wales. By 1910 the Massachusetts figure had increased to fifty-one years, showing a gain of eleven years in a half century. By 1920 the expectation of life in Massachusetts had reached fifty-five years, or a gain of more than fifteen in sixty-five calendar years. In the Registration Area of the United States there has been an increase of six years between 1901 and 1920.

Turning to the mortality in age groups, Dublin says that the infant mortality in the Registration Area dropped in 1921 to 76.0 per 1,000 births. In New Zealand in 1920 it was below 50.0, and there are a large number of cities in the United States, some of good size, where the infant mortality at the present time is below 40.0 per 1,000 births. Dublin assumes that it is entirely feasible to reduce the infant mortality to 38.2 per 1,000 births; indeed, he thinks that this is not an unreasonable figure and he is inclined to the opinion that the reduction might be still greater if more attention is paid to the prevention of infant mortality during the first month. The detection and treatment of syphilis in the mother, the control of the toxemias of pregnancy, and the provision for skillful obstetrics would do much for the mothers and would greatly reduce the death rate during the first month of life. Dublin is inclined to think that an infantile death rate of 20.0 per 1,000 births is possible, but he has assumed the higher rate of 38.2.

This author assumes a death rate during the second, third and fifth years of life of between 34.0 and 40.0 per cent of the actual mortality rates as shown in the census of 1910, and he assumes the possibility of reaching a mortality rate of 4.2 per 1,000 for the third year of life. In New Zealand nearly fifteen years ago this rate was 5.3. During the third year of life virtually two-thirds of the entire mortality is due to such infections as typhoid fever, diarrhea, measles, scarlet fever, whooping cough and diphtheria, all of which are more or less under human control.

Dublin assumes that during the period between ten and sixty the mortality can be reduced to one-half the figure prevailing in 1910. He says that his assumed values are for the most part only 20.0 per cent below those of New Zealand in recent years. In that country, as in ours, there is still much preventable mortality from tuberculosis in the working period of life. There is also marked mortality from occupational and other accidents, most of which might be avoided. Dublin says that his assumed rates between ten and sixty are very close to those attained by the best life insurance companies

in their current ordinary experience; indeed, at some ages the actual insurance experience is even lower than the values here named. An investigation into the mortality of a group of ordinary policyholders of the Metropolitan Life Insurance Company who have availed themselves of the privilege of examinations over five years ago showed at the end of the period a mortality 28.0 per cent lower than that of the best control group used in comparison.

Since 1911 tuberculosis has declined 50.0 per cent among those insured in the Metropolitan Company and almost as much in the general population of several progressive cities. Not only is this disease rapidly declining in mortality, but the peak of maximum rate by age is constantly being pushed forward into the later years of life. Unfortunately, we are not doing so well with the diseases which depend upon personal hygiene and the general care of the human body. Death rates from the so-called degenerative diseases, like those of the heart and kidney, and other diseases affecting middle life and old age, are not declining. We are making little headway along these lines. These diseases cause heavy losses to the community, because they involve those still in their prime and at the height of their productivity. Heart disease, Bright's disease, and cerebral hemorrhage curtail the expectation of life about four years. Further reduction in the incidence of infectious diseases, such as typhoid fever and diphtheria will bring important results so far as these degenerative diseases are concerned. It is reasonable to expect that an appreciable part of the 50.0 per cent mortality reduction postulated by Dublin in his hypothetical life table will be accomplished through these means. Much good will be done during the productive period of life by improving conditions under which individuals work. Occupational accidents are responsible each year in the United States for at least 15,000 deaths; most of these among men in their prime. In his conclusions Dublin makes no allowance for improvement in the possible saving of life from cancer; neither does he assume that there will be any marked decrease in senile diseases.

From his conclusions, Dublin thinks it within reason to look forward to the prolongation of human life in this country, which is now about fifty-five years, to sixty-five years. The total death rate would be reduced from 13.0 to 8.6 per 1,000. In other words, an extension of the expectation of life by ten years is within the range of accomplishment. The greatest reduction would be in the infant mortality, which would be to one-third that now existing. A reduction during the first year of life, such as provided for in the table, would mean a saving of 126,000 infants. During the first five years of life there would be 171,000 children saved. Between the ages of ten and sixty a reduction, regarded as reasonable, would save 250,000 lives. These figures show strikingly how great are the stakes involved in the future of medicine.

It should be stated that Dublin has figured out these possibilities without calling to his aid any great medical discovery. If an advance could be made in the treatment of heart disease and kidney disease comparable with that recently made in the treatment of syphilis the expectation of life might be considerably advanced above the figures given. —*V. C. V.*

Ray Therapy for Deafness and Tinnitus

A PROMINENT roentgenologist has referred to the x-ray treatment of defective hearing as the latest and favorite indoor sport among roentgenologists. The first serious attempt to treat ear conditions by this method was made by Joulin, who in 1908, reported results in ten cases of otosclerosis. There was some improvement in the tinnitus and some apparent improvement in voice perception. No improvement could be demonstrated by objective tests. The next observer, Ortloff, in 1913, published his conclusions on the treatment of ten cases of tinnitus and deafness, in whom he had observed slight improvement, invariably followed by relapse. Siebenman, after a study covering a period of several years, concluded that the results are only in a small degree encouraging. In this country, Stokes in 1920 reported startling achievements with this method of treatment. Since then great numbers of individuals with tinnitus and deafness from whatever source have received varying amounts of x-ray treatment.

Tinnitus may accompany a number of pathological processes, but it usually indicates some local organic change such as labyrinthine disease, suppurative otitis media, tuberculosis, disease of the auditory nerve, or otosclerosis. Although there are many theories the cause of otosclerosis is not known. In the later stages there is always considerable associated bony deposit around the articulation of the stapes. Once it has occurred, this proliferative change can scarcely be alleviated by x-ray treatment. If the original cause of otosclerosis be amenable to ray therapy, we might anticipate some success in delaying or in stopping the progress of the disease. Little more however could be accomplished.

Raynal suggests that the x-ray treatment of otosclerosis be supplemented by repeated catheterization of the Eustachian tube, to ultimately produce improved mobility of the ossicles.

Tinnitus, on the other hand, is often due to chronic catarrhal otitis media, which in turn results from obstruction or other disease in the nasopharynx. It is conceivable that removal of the latter with resultant greater patency of tubes and improved drainage of the middle ear may give relief from head noises. The greater the chronicity with its resulting changes, the less will be the probability of improvement.

Kinney has found that in cases associated with disease in the nasopharynx, forty per cent obtained permanent decrease in the tinnitus and marked improvement in the hearing, both subjectively and to objective tests. Although improvement was sometimes noted in otosclerosis and in chronic otitis media, the results were on the whole less satisfactory.

Jarvis classifies the cases of impaired hearing treated with the roentgen ray as follows. The first class includes children and young adults whose impairment is often explained by the presence of a mass of adenoid tissue in the nasopharynx. The second includes individuals usually between the years of twenty-five and fifty who are subject to frequent head colds, complain of a more or less constant catarrhal discharge from the throat, and

at frequent intervals of a stuffiness in the ears with accompanying deafness. They frequently complain of tinnitus. In the third class are those, usually advanced in years, whose chief symptom is deafness.

In the first class obstruction to respiration is the prominent symptom. In the second throat symptoms exist, while in the third the complaint is only of impaired hearing. Jarvis obtained best results in class two. In them he often finds large middle and inferior turbinates and lymphoid hypertrophy running up either side of the pharynx behind the posterior tonsillar pillars. Relief from tinnitus in the second group was quite constant. This was not uniformly the case in group three.

McCoy, following Stokes' method of treatment, obtained marked improvement in audition in nine out of thirty-five cases of chronic catarrhal otitis media, in none out of two cases of chronic purulent otitis media, in one of two cases of residual purulent otitis media and in two out of six cases of otosclerosis. In some additional cases there was slight improvement, more marked among those suffering from chronic catarrhal otitis media.

He observed a greater patency of the Eustachian tubes in those cases previously affected with a tendency to stenosis thereof. Two individuals believed that they had experienced a decided improvement in the sense of smell. Tinnitus, which was present in nearly all, was stopped in six and alleviated in ten.

Several workers have experienced no satisfactory results, particularly as far as objective tests for improvement were concerned. Hickey, who has treated a number of individuals, found no objective benefit, although many seemed to think that they were some better. Law reports similar results and remarks on the difficulty of making the voice tone of equal intensity twice in succession, especially when one is anxious that the patient should hear better. Also there is a certain psychological element in the patient's attitude. Fowler again found no objective improvement although the patients were usually of the opinion that they could hear better.

Raynal reports considerable relief from attacks, without complete cure, in a case of Meniere's disease.

The work of Kinney and of Jarvis, who in their classifications, have brought out the importance of nasopharyngeal conditions in those cases successfully treated, will aid in obtaining results, by enabling the physician to select such cases as may *a priori* be expected to respond satisfactorily.

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—*W. T. V.*

A Brief Sketch of the Early History of Industrial Hygiene

ANCIENT Greek and Roman medical men were not ignorant of the influence of occupation upon the causation of disease. According to Pliny, it was the custom even long before his time, for those working in metals, especially in lead, to protect themselves from inhaling the dust by wearing respirators. In 1557 Agricola published a book, entitled, "De re metallica" in which he pointed out the fact that workers in dusty occupations are prone to asthma and tuberculosis. In 1700 Ramazzini, of Padua, wrote most learnedly and extensively concerning occupational diseases. In his book, "De morbis artificium diatriba," he dealt with dusty occupations and showed that those passing their lives in such occupations furnish an unusually large percentage of cases of tuberculosis. Plumbism, with its most marked signs and symptoms, has been recognized and its cause correctly determined from the earliest historical times.

In the reign of Elizabeth (early seventeenth century) Parliament enacted a law permitting persons who desired to do so to establish houses in which apprenticed children might be instructed in spinning and weaving. This was actually the beginning of the factory system in England. Orphans and other destitute children were apprenticed to the owners of these primitive factories. Children of all ages were employed in these institutions and, in some at least, the working hours covered fourteen of each day. It seems that the framers of this early law were actuated, in part at least, by good motives. They desired to give to orphans and other destitute children an opportunity to earn a living. Long before this time spinning and weaving had occupied a large proportion of the time of the industrial poor, but this work was done in small, crowded cottages and often in cellars.

The poor-law act of 1601, whereby apprenticed children could be instructed in spinning and weaving, gradually grew into the great cotton mills of England. In the early days these mills were located in the country where water power could be obtained. The discovery of the steam engine and its use in factories, which occurred about 1785, led to the rapid growth of such manufacturing centers as Manchester and greatly complicated provision for sanitation in the factories. The imports of raw cotton into England stood in 1775 at 5,000,000 pounds. By 1831 this had grown, according to Hope, to 273,000,000 pounds.

From the beginning of the schools for instruction in spinning and weaving, apprenticed children were frequently exploited in a shameful way. They were fed niggardly, housed when not at work under insanitary surroundings, and during working hours which, as we have seen, often extended to fourteen or fifteen a day, they were densely crowded, subjected to infection, and exposed to an environment in which they could not retain health nor resist disease. It was not, however, until 1784, nearly 200 years after the system had been inaugurated that serious complaint was made. This was first voiced by Percival, who reported infectious outbreaks among children employed in certain factories in Lancashire. For eleven years

Percival continued his criticism of the insanitary conditions under which apprenticed children lived and worked in the cotton mills. In 1795 Percival and associates constituted themselves into a local board of health and demanded that there should be legislative protection for the children employed in factories. In 1802 Peel secured the passage of a bill under the title of the "Health and Morals of Apprentices Act." This Act forbade the employment of children for more than twelve hours a day, ordered that provision for elementary education should be made, that the children should be supplied with sufficient clothing, and that the factories should be properly ventilated and whitewashed at least twice a year. The Act, however, was not a success, for two reasons. In the first place, it created a board of inspectors or visitors, which board consisted of two, one of whom should be a justice of the peace and the other a clergyman. These boards did nothing, largely because their members knew nothing concerning the sanitary conditions of factories or the effects of insanitary conditions upon health. In the second place, the Act was confined to the protection of apprenticed children. It was very easy for the owners of these factories to get rid of their apprentices and introduce in their places "free children." They found parents willing that their children should live under any conditions, provided their work contributed to the support of the family. It was not until 1815 that Peel secured the passage of an act limiting the working day to ten hours, applicable to all children, apprenticed and free, employed in cotton mills. In 1832 Thackrah, a Leeds surgeon, published his book, "The Diseases of Tradesmen," which discussed the relation between occupation and disease. In 1833 an act was passed providing for industrial inspection by qualified men, employed by and under the direction of the general government board of health.

It must not be inferred from what we are saying that all mill owners were disregardful of the welfare of their employes. There were some who were ready, even at a financial sacrifice, to improve the sanitary conditions of their factories, restrict the hours of child labor, and provide for elementary education. In 1842 Parliament passed an act excluding women and children of all ages from working in the mines, and two years later, provision for inspectors was extended to all textile factories and a medical examination of all under sixteen years of age was required before employment. The same act prohibited the employment of children under eight years of age and restricted female labor in cotton mills. The introduction of steam-driven machinery had greatly increased the danger of accident and provision for wire guards was made. In 1860 a bill was passed placing bleaching and dyeing works under the factories act. This bill, however, was defective and did not become beneficially operative until ten years later. Special provision was made against the dangers in glazing pottery and in the manufacture of matches. Strange to say, no effective legislation for protecting the workman engaged in the manufacture of white lead was enacted until 1883, and it was not until 1891 that special provision was

made applicable to every industry which, in the opinion of the Secretary of State, might injuriously affect the health of workers.

After the factories had received fairly satisfactory attention so far as the health and welfare of workers were concerned, there grew up, first in the east end of London, what has been known as the "sweat shop," the evils of which have extended to every manufacturing city in the world. Most of the industrial acts had defined a factory as any place in which fifty or more workers are employed. In the sweat shop the provisions of these laws were avoided by the performance of labor in the home. The Workman's Compensation Act, which became a law in England in 1897 and in this country by the several states later, has been of substantial benefit to the health of the working man; indeed, after trial it seems to be approved by the majority of employers.

In this country we have closely followed England in the development of industrial hygiene. In former times the manufacturer supposed the more hours a day his employes worked the more he was getting for his daily wage. The part played by fatigue was unknown and not even suspected by the manufacturer of fifty years ago. We may quite safely predict that the required working hours in our manufacturing establishments will be further decreased and that the conditions of life under which work is done will be improved. —*V. C. V.*

BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, 404 Professional Building, Richmond, Va.)

*Sollmann's Pharmacology**

THE second edition of this book continues the high standard set by the first. It is arranged with two sets of type, large for general discussions and small for more detailed information. In this way each drug may be studied either minutely or with regard only to the prominent characteristics. Side headings are found at the beginning of nearly every paragraph. As a consequence of these two methods of treatment the book is most valuable for reference work. At the same time, the author has been forced thereby to discard attempts to make the discussions rhetorically sequential. There is usually a slight break in the line of thought at each side heading. From the nature of the work this could scarcely be avoided.

The work is splendidly done throughout. Among the chapters dealing with newer subjects are particularly those on colloidal solutions, electrolysis, osmosis, synthetic drugs, anaphylaxis and the various war gases. The author's

*A Manual of Pharmacology and Its Applications to Therapeutics and Toxicology. By Torald Sollmann, M.D., Professor of Pharmacology and Materia Medica in the School of Medicine of Western Reserve University, Cleveland. Second edition, entirely reset. Cloth. Price $5.50. Pp. 1006. Philadelphia and London. W. B. Saunders Company. 1922.

discussion of the pharmacologic action and therapeutic use of alcohol is of particular interest at this time.

A comprehensive bibliography is arranged at the end, and thereby does not interfere with the reading.

Microscopic Examination of Foods and Drugs†

SOLLMANN, in his manual of Pharmacology writes, "The appearance, size and arrangement of the cells and their inclosures (starch grains, etc.) is often the most important aid in identifying a drug, particularly when in the form of powder. The pharmacopeias therefore include microscopic descriptions, whenever these are important."

Greenish's book deals with this method of examination.

Written primarily for those interested in pharmacy and drug and food adulterants, it deals entirely with the microscopic examination and identification of starches, hairs, fibers, spores, glands, woods, stems, leaves, flowers, bark, seeds, fruits, rhizomes and roots. There is a chapter on the adulterants of powdered foods and drugs and a general scheme for the microscopic examination of powders. At the end of each heading the author describes a method for the identification of an unknown powder. The survey of the subject is most comprehensive.

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*Modern Microscopy**

THE first ninety-one pages of this book deal with the microscope, its construction and use. The physics of light, etc., is discussed in great detail. Various types of microscopes are described. The second portion deals with the use of the microscope in medicine, public health, in the study of tropical diseases, in histology, geology, engineering, agriculture, natural history, etc. But a few pages are devoted to each. The last chapter describes methods of mounting common objects for examination.

There can be no doubt but that when the book was first written in 1893, and the microscope was still much of an innovation, such a book would have been extremely valuable. However, as time goes on, the field of usefulness for a scientific book dealing with the present day microscope per se is gradually dwindling.

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†The Microscopical Examination of Foods and Drugs. By Henry G. Greenish, F.I.C., the University of London; Director of the Pharmacy Research Laboratory; Docteur (H.C.) De L'Universite De Paris; Hamburg Gold Medallist, 1917; Joint Editor of the British Pharmacopeia, 1914; Late Member of the Board of Examiners of the Pharmaceutical Society of Great Britain; Late External Examiner in Materia Medica and Pharmacy to the University of Birmingham. A Practical Introduction to the Methods Adopted in the Microscopical Examination of Foods and Drugs, in the Entire, Crushed and Powdered States. Third edition. Cloth. Price $4.50. Pp. 386 with 209 illustrations. Philadelphia: P. Blakiston's Son & Co., 1012 Walnut Street. 1923.

*Modern Microscopy. A Handbook for Beginners and Students. By M. I. Cross and Martin J. Cole. Lecturer in History at Cooke's School of Anatomy. Fifth edition. Revised and rearranged by Herbert F. Angus. Cloth. Price $3.50. Chicago Medical Book Co., Chicago, Ill. Pp. 315 with numerous illustrations and diagrams. 1923.